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## (54) Nucleic acid probes complementary to Human Papillomavirus nucleic acid and related methods and kits

(57) The present invention describes oligonucleotides targeted to HPV Type 16 and/or Type 18 nucleic acid sequences which are particularly useful to aid in detecting HPV type 16 and or 18. The oligonucleotides can aid in detecting HPV Type 16 and/or Type 18 in different ways such as by acting as hybridization assay probes, helper probes, and/or amplification primers.

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## Description

### Field of the Invention

This invention relates generally to nucleic acid probes complementary to Human Papillomavirus (hereafter "HPV") nucleic acids, methods of using such probes, and kits containing such probes. In particular, different types of oligonucleotide probes are described (including hybridization assay probes, helper oligonucleotides and amplification oligonucleotides) which are useful for detecting HPV Type 16 and/or Type 18 in a test sample, such as a vaginal swab, a cervical swab, a urethral swab, a tissue sample, a body fluid or an experimental solution.

### Background of the Invention

The following description of the background of the invention and references cited therein are not admitted to be prior art to the present invention.

Papillomaviruses are small DNA viruses. These viruses are associated with and/or thought to be the causative agent of a range of benign conditions (including benign lesions and benign tumors). Papillomaviruses have also been associated with malignancies such as squamous cell carcinoma in patients having the autosomal disease epidermodysplasia verricruciformis, and with genital cancers in both males and females.

There have now been at least 59 different types of HPV characterized, (see Manual of Clinical Microbiology; 998-1000; 5th ed. American Soc. for Microbiol. 1991). The genome of different HPV variants appears to be similar between all types (Van Ranst et al., J. Gen. Vir., 73:2653-60, 1992). Nonetheless, HPVs have been subject to differential typing, based on differences in the DNA sequences of different strains of the virus (Id.).

Among those HPV types associated with genital cancers are HPV types 16, 18, 31, 33 and 35. These five strains collectively are found in over 80% of all cervical tumors, suggesting a causative role.

Antigen detection of HPV types 16 and 18 has been described, but it is reported that commercially available sera react with antigens shared by all papillomaviruses (Roman and Fife, Clin. Microbiol. Rev. 2:166-190, 1989). In addition, the percentage of antigen-positive specimens is reported to decrease as the severity of the disease increases from mild dysplasia to carcinoma in situ, to invasive carcinoma (Id.).

*In vivo*, HPV DNA is found both episomally and integrated in the host genome. The HPV genome contains open reading frames encoding from 8 to 10 proteins, although not all of these proteins have been identified. Many of these open reading frames have been designated with the prefixes E or L, referring to "early" or "late" transcription events, although not all of those designated "early" are actually transcribed early, and vice versa.

Descriptions of certain primers and oligonucleotide probes for the detection of the E6 region of HPV types 16 and 18 are provided in Lucotte *et al.*, Mol. Cell. Probes 7:339-344, 1993; De Britton *et al.*, Obst. Gynec. 81:19-24, 1993; Nuovo, *et al.*, Am. J. Pathol. 138:53-58, 1991; Van der Velde *et al.*, J. Med. Virol. 36:279-282, 1992; Thompson *et al.*, J. Med. Virol. 36:54-6, 1992; Cornelissen *et al.*, J. Gen. Virol. 71:1243-1246, 1990, Hus and McNicol, Mol. Cell. Probes 6:459-466, 1992; Sang and Barbosa Virol. 189:448-455, 1992; Joseph, European Publication Number O 477 972; Joannes *et al.*, PCT Publication Number WO 93/02217; Emery *et al.*, International Publication Number WO 92/01815; Hendricks, International Publication Number WO 91/08312, International Application Number PCT/US90/07057; Manos *et al.*, U.S. Patent No. 5,182,377; Herzog, *et al.*, U.S. Patent No. 4,983,728; Schwartz and Adams, International Publication Number WO 89/02934; George and Groff, International Publication Number WO 89/09940; Nur *et al.*, International Publication Number WO 92/14847; Mazzatente *et al.*, European Patent Publication Number EPO 489 442; Shimada *et al.*, European Patent Publication Number EPO 402 132; and Morris *et al.*, International Publication Number WO 88/06634; all of which are hereby incorporated herein by reference in their entirety (including drawings).

### Summary of the Invention

The present invention features oligonucleotides useful for detecting HPV Type 16 and/or Type 18 , methods of making and using these oligonucleotides, and kits containing the oligonucleotides. The featured oligonucleotides include hybridization assay oligonucleotides, amplification oligonucleotides, and helper oligonucleotides. The different oligonucleotides can aid in the detection of HPV Type 16 and/or Type 18 in different ways.

Hybridization assay probe oligonucleotides are targeted to HPV Type 16 and/or Type 18 regions and preferably are labeled. These oligonucleotides are particularly useful for distinguishing between HPV Type 16 and/or Type 18 variants from other HPV variants, including HPV 6, 11, 31, 33, 35, 39, 45, 51, 52, or 58. The target region for the hybridization assay oligonucleotides includes nucleic acids specifically found in HPV Type 16 and/or Type 18, or a nucleic acid sequence complimentary thereto. Complimentary nucleic acid can be produced using standard well known nucleic acid amplification techniques.

The amplification primers can be used to initiate amplification reactions using HPV target nucleic acid. The primers

are designed to hybridize to a region of the target nucleic acid 3' of a target region. The primers can be used to initiate amplification synthesizing copies of nucleic acid complementary to the target region. Different types of amplification can be performed depending upon the amplification primer which is utilized. For example, pairs of amplification primers hybridizing to a region 3' of the target sequence and to a region 3' of a complimentary target sequence can be used in PCR amplification. Primers which hybridize to a region 3' to the target sequence which have a promoter sequence recognized by a promoter (such as those used by bacteriophage T7, T3 or SP-6) can be used to synthesize multiple copies of nucleic acid complimentary to the target sequence.

Helper probes are particularly useful for facilitating the hybridization of a hybridization assay oligonucleotide to its target sequence. Helper probes aid in altering the secondary structure of nucleic acid in and around the target region. The use of helper probes is describe by Hogan and Milliman, U.S. Patent No. 5,030,557, which is incorporated by reference herein in its entirety including any drawings. Also featured are probe mixes containing one or more labeled probes and at least one helper probe for use in hybridization assays for the detection of HPV and methods of detecting and amplifying HPV nucleic acids.

The probes, their complements or RNA equivalents, can be used to distinguish HPV Type 16 and/or Type 18 from closely related phylogenetic neighbors, by preferentially hybridizing to an HPV Type 16 and/or Type 18 target nucleic acid sequence region under selective hybridization assay conditions. The hybridization assay probes disclosed herein are particularly useful for detecting the presence of HPV Type 16 and/or Type 18 and/or for determining the quantity of HPV Type 16 and/or Type 18 present in a test sample, e.g., samples of sputum, urine, blood, tissue sections, urogenital secretions, urogenital swabs and other clinical samples.

Hybridization assay oligonucleotide probes contain a nucleotide sequence perfectly complementary, or substantially complementary, to an HPV target sequence. In addition to having a region designed to distinguish between HPV Type 16 and/or Type 18 on the one hand and different HPV variants on the other hand, hybridization assay probes can also have one or more additional nucleic acid sequences which are complementary to additional stretches of an HPV target nucleic acid or non-complementary nucleic acid sequences. For example, the additional sequences can be complementary to both HPV Type 16 and/or Type 18 and other HPV variants, they can be non-complementary to HPV Type 16 and/or Type 18, or HPV variants, or they can even have a slightly higher degree of complementarity to the HPV variants as long as the hybridization probe is able to distinguish HPV Type 16 and/or Type 18 from other HPV variants such as HPV types 6, 11, 31, 33, 35, 39, 45, 51, 52, or 58.

Hybridization assay probes are sufficiently complementary to nucleic acids containing a target sequence to form a stable and detectable hybrid probe:target duplex under stringent hybridization assay conditions. A hybridization assay probe is preferably between 10 and 100 nucleotides in length, more preferably between 14 and 50 nucleotides in length. Even more preferably the probe is between 18 and 40 nucleotides in length. Hybridization assay probes are preferably labeled with a reporter group moiety such as a radioisotope, a fluorescent moiety, a chemiluminescent moiety, an enzyme, or a ligand incorporated into the probe. The moiety can be used to detect or confirm probe hybridization to its target sequence. A hybridization assay probe is an oligonucleotide which can distinguish HPV type 16 and/or 18 from other HPV types and or common body flora, by preferentially hybridizing to an HPV type 16 and/or 18 target nucleic acid sequence region under stringent hybridization assay conditions.

## I. Hybridization Assay Probes

Thus, in one aspect the invention features a hybridization asay probe containing an oligonucleotide able to hybridize to an HPV Type 16 and/or Type 18 target nucleic acid to form a detectable target:probe duplex under selective stringency hybridization conditions, but which preferably will not form a detectable non-target:probe duplex with nucleic acids from HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, and/or 58. The oligonucleotide comprises a sequence of nucleic acids which is at least 70%, (preferably 80%, more preferably 90%, and most preferably 100% complementary) to a target sequence of 10 or more contiguous nucleotides present in a target region. The target regions can be better understood with reference to Table A below.

Table A

| Sequence | Seq. ID No. | Type - HPV 16, - HPV 18, - Probe - Primer - Helper | Sub-Type 1, 2 or 3 | DNA or RNA |
|---|---|---|---|---|
| GACATTATTG TTATAGTTTG TATGGAAC | 1 | HPV 16 primer | 2 | DNA |
| GTTCCATACA AACTATAACA ATAATGTC | 2 | | | DNA |
| GACAUUAUUG UUAUAGUUUG UAUGGAAC | 3 | | | RNA |
| GUUCCAUACA AACUAUAACA AUAAUGUC | 4 | | | RNA |
| | | | | |
| GAACAGCAAT ACAACAAACC GTTGTGTG | 5 | HPV 16 probe | 3 | DNA |
| CACACAACGG TTTGTTGTAT TGCTGTTC | 6 | | | DNA |
| GAACAGCAAU ACAACAAACC GUUGUGUG | 7 | | | RNA |
| CACACAACGG UUUGUUGUAU UGCUGUUC | 8 | | | RNA |
| | | | | |
| GACGTGAGGT GTATTAACTG TCAAAAG | 9 | HPV 16 probe | 2 | DNA |
| CTTTTGACAG TTAATACACC TCACGTC | 10 | | | DNA |
| GACGUGAGGU GUAUUAACUG UCAAAAG | 11 | | | RNA |
| CUUUUGACAG UUAAUACACC UCACGUC | 12 | | | RNA |
| | | | | |
| CCATGCATGA TTACAGCTGG GTTTCTC | 13 | HPV 16 primer | 2 | DNA |
| GAGAAACCCA GCTGTAATCA TGCATGG | 14 | | | DNA |
| CCAUGCAUGA UUACAGCUGG GUUUCUC | 15 | | | RNA |
| GAGAAACCCA GCUGUAAUCA UGCAUGG | 16 | | | RNA |

| Sequence | Seq. ID No. | Type - HPV 16, - HPV 18, - Probe - Primer - Helper | Sub-Type 1, 2 or 3 | DNA or RNA |
|---|---|---|---|---|
| | | | | |
| TACGTGTTCT TGATGATCTC ACGTCG | 17 | HPV 16 probe | 2 | DNA |
| CGACGTGAGA TCATCAAGAA CACGTA | 18 | | | DNA |
| UACGUGUUCU UGAUGAUCUC ACGUCG | 19 | | | RNA |
| CGACGUGAGA UCAUCAAGAA CACGUA | 20 | | | RNA |
| | | | | |
| GTGTGTACTG CAAGCAACAG TTACTG | 21 | HPV 16 primer | 2 | DNA |
| CAGTAACTGT TGCTTGCAGT ACACAC | 22 | | | DNA |
| GUGUGUACUG CAAGCAACAG UUACUG | 23 | | | RNA |
| CAGUAACUGU UGCUUGCAGU ACACAC | 24 | | | RNA |
| | | | | |
| CTTTTGACAG TTAATACACC TCACG | 25 | HPV 16 probe | 3 | DNA |
| CGTGAGGTGT ATTAACTGTC AAAAG | 26 | | | DNA |
| CUUUUGACAG UUAAUACACC UCACG | 27 | | | RNA |
| CGUGAGGUGU AUUAACUGUC AAAAG | 28 | | | RNA |
| | | | | |
| AAAGTCATAT ACCTCACGTC GC | 29 | HPV 16 probe | 2 | DNA |
| GCGACGTGAG GTATATGACT TT | 30 | | | DNA |
| AAAGUCAUAU ACCUCACGUC GC | 31 | | | RNA |

| Sequence | Seq. ID No. | Type - HPV 16, - HPV 18, - Probe - Primer - Helper | Sub-Type 1, 2 or 3 | DNA or RNA |
|---|---|---|---|---|
| GCGACGUGAG GUAUAUGACU UU | 32 | | | RNA |
| | | | | |
| GAAACCCAGC TGTAATCATG C | 33 | HPV 16 probe | 1 | DNA |
| GCATGATTAC AGCTGGGTTT C | 34 | | | DNA |
| GAAACCCAGC UGUAAUCAUG C | 35 | | | RNA |
| GCAUGAUUAC AGCUGGGUUU C | 36 | | | RNA |
| | | | | |
| GATCATCAAG AACACGTAG | 37 | HPV 16 primer | 1 | DNA |
| CTACGTGTTC TTGATGATC | 38 | | | DNA |
| GAUCAUCAAG AACACGUAG | 39 | | | RNA |
| CUACGUGUUC UUGAUGAUC | 40 | | | RNA |
| | | | | |
| GGAACTGAAC ACTTCACTGC AAGACATAGA AATAACC | 41 | HPV 18 primer | 3 | DNA |
| GGTTATTTCT ATGTCTTGCA GTGAAGTGTT CAGTTCC | 42 | | | DNA |
| GGAACUGAAC ACUUCACUGC AAGACAUAGA AAUAACC | 43 | | | RNA |
| GGUUAUUUCU AUGUCUUGCA GUGAAGUGUU CAGUUCC | 44 | | | RNA |
| | | | | |

| Sequence | Seq. ID No. | Type - HPV 16, - HPV 18, - Probe - Primer - Helper | Sub-Type 1, 2 or 3 | DNA or RNA |
|---|---|---|---|---|
| GGAAAAACTA ACTAACACTG GGTTATACAA T | 45 | HPV 18 probe | 1 | DNA |
| ATTGTATAAC CCAGTGTTAG TTAGTTTTTC C | 46 | | | DNA |
| GGAAAAACUA ACUAACACUG GGUUAUACCA U | 47 | | | RNA |
| AUUGUAUAAC CCAGUGUUAG UUAGUUUUUC C | 48 | | | RNA |
| | | | | |
| CATAGAAATA ACCTGTGTATA TTGCAAG | 49 | HPV 18 primer | 2 | DNA |
| CTTGCAATAT ACACAGGTTAT TTCTATG | 50 | | | DNA |
| CAUAGAAAUA ACCUGUGUAUA UUGCAAG | 51 | | | RNA |
| CUUGCAAUAU ACACAGGUUAU UUCUAUG | 52 | | | RNA |
| | | | | |
| GACATTATTC AGACTCTGTGT ATGGAG | 53 | HPV 18 primer | 2 | DNA |
| CTCCATACAC AGAGTCTGAAT AATGTC | 54 | | | DNA |
| GACAUUAUUC AGACUCUGUGU AUGGAG | 55 | | | RNA |
| CUCCAUACAC AGAGUCUGAAU AAUGUC | 56 | | | RNA |
| | | | | |
| GCAAGACAGT ATTGGAACTT ACAGAG | 57 | HPV 18 probe | 3 | DNA |
| CTCTGTAAGT TCCAATACTG TCTTGC | 58 | | | DNA |
| GCAAGACAGU AUUGGAACUU ACAGAG | 59 | | | RNA |
| CUCTGUAAGU UCCAAUACUG UCUUGC | 60 | | | RNA |

| Sequence | Seq. ID No. | Type - HPV 16, - HPV 18, - Probe - Primer - Helper | Sub-Type 1, 2 or 3 | DNA or RNA |
|---|---|---|---|---|
| | | | | |
| CCTGTGTATA TTGCAAGACAG TATTG | 61 | HPV 18 helper | 2 | DNA |
| CAATACTGTC TTGCAATATAC ACAGG | 62 | | | DNA |
| CCUGUGUAUA UUGCAAGACAG UAUUG | 63 | | | RNA |
| CAAUACUGUC UUGCAAUAUAC ACAGG | 64 | | | RNA |
| | | | | |
| GAACTTACAG AGGTATTTGA ATTTGC | 65 | HPV 18 probe | 3 | DNA |
| GCAAATTCAA ATACCTCTGT AAGTTC | 66 | | | DNA |
| GAACUUACAG AGGUAUUUGA AUUUGC | 67 | | | RNA |
| GCAAAUUCAA AUACCUCUGU AAGUUC | 68 | | | RNA |
| | | | | |
| CAACCGAGCA CGACAGGAACG AC | 69 | HPV 18 primer | 2 | DNA |
| GTCGTTCCTG TCGTGCTCGGT TG | 70 | | | DNA |
| CAACCGAGCA CGACAGGAACG AC | 71 | | | RNA |
| GUCGUUCCUG UCGUGCUCGGU UG | 72 | | | RNA |
| | | | | |
| CCAACGACGC AGAGAAACAC AAG | 73 | HPV 18 probe | 2 | DNA |
| CTTGTGTTTC TCTGCGTCGT TGG | 74 | | | DNA |
| CCAACGACGC AGAGAAACAC AAG* | 75 | | | RNA |

| Sequence | Seq. ID No. | Type - HPV 16, - HPV 18, - Probe - Primer - Helper | Sub-Type 1, 2 or 3 | DNA or RNA |
|---|---|---|---|---|
| CUUGUGUUUC UCUGCGUCGU UGG | 76 | | | RNA |
| | | | | |
| CTTACAGAGG TGCCTGCGGT GC | 77 | HPV 18 probe | 3 | DNA |
| GCACCGCAGG CACCTCTGTA AG | 78 | | | DNA |
| CUUACAGAGG UGCCUGCGGU GC | 79 | | | RNA |
| GCACCGCAGG CACCUCUGUA AG | 80 | | | RNA |
| | | | | |
| GAACTTACAG AGGTGCCTGC GG | 81 | HPV 18 probe | 3 | DNA |
| CCGCAGGCAC CTCTGTAAGT TC | 82 | | | DNA |
| GAACUUACAG AGGUGCCUGC GG | 83 | | | RNA |
| CCGCAGGCAC CUCUGUAAGU UC | 84 | | | RNA |
| | | | | |
| CAGGACACAG TGGCTTTTGA C | 85 | HPV 16 primer | 3 | DNA |
| GTCAAAAGCC ACTGTGTCCT G | 86 | | | DNA |
| CAGGACACAG UGGCUUUUGA C | 87 | | | RNA |
| GUCAAAAGCC ACUGUGUCCU G | 88 | | | RNA |
| | | | | |
| GCTTTTTGTC CAGATGTCTT TGC | 89 | HPV 16 primer | 2 | DNA |
| GCAAAGACAT CTGGACAAAA AGC | 90 | | | DNA |

| Sequence | Seq. ID No. | Type - HPV 16, - HPV 18, - Probe - Primer - Helper | Sub-Type 1, 2 or 3 | DNA or RNA |
|---|---|---|---|---|
| GCUUUUUGUC CAGAUGUCUU UGC | 91 | | | RNA |
| GCAAAGACAU CUGGACAAAA AGC | 92 | | | RNA |
| | | | | |
| GCAATGTAGG TGTATCTCCA TGC | 93 | HPV 16 primer | 1 | DNA |
| GCATGGAGAT ACACCTACAC CGC | 94 | | | DNA |
| GCAAUGUAGG UGUAUCUCCA UGC | 95 | | | RNA |
| GCAUGGAGAU ACACCUACAC CGC | 96 | | | RNA |
| | | | | |
| AATTTAATAC GACTCACTAT AGGGAGA | 97 | T7 Polymerase primer | 1 | DNA |
| TCTCCCTATA GTGAGTCGTA TTAAATT | 98 | | | DNA |
| AAUUUAAUAC GACUCACUAU AGGGAGA | 99 | | | RNA |
| UCUCCCUAUA GUGAGUCGUA UUAAAUU | 100 | | | RNA |
| | | | | |
| TCGTTTTTCATTAAGGTGTCTAAGTTTTTCTGCTGGATT C | 101 | HPV 18 primer | 1 | DNA |
| GAATCCAGCAGAAAAACTTAGACACCTTAATGAAAAA CGA | 102 | | | DNA |
| UCGUUUUUCAUUAAGGUGUCUAAGUUUUUCUGC UGGAUUC | 103 | | | RNA |
| GAAUCCAGCAGAAAAACUUAGACACCUUAAUGAAA AACGA | 104 | | | RNA |

| Sequence | Seq. ID No. | Type - HPV 16, - HPV 18, - Probe - Primer - Helper | Sub-Type 1, 2 or 3 | DNA or RNA |
|---|---|---|---|---|
| | | | | |
| GCAATGTTGC CTTAGGTCCA TGC | 105 | HPV 18 primer | 2 | DNA |
| GCATGGACCT AAGGCAACAT TGC | 106 | | | DNA |
| GCAAUGUUGC CUUAGGUCCA UGC | 107 | | | RNA |
| GCAUGGACCU AAGGCAACAU UGC | 108 | | | RNA |
| | | | | |
| CGGTTTCTGG CACCGCAGGC AC | 109 | HPV 18 primer | 2 | DNA |
| GTGCCTGCGG TGCCAGAAAC CG | 110 | | | DNA |
| CGGUUUCUGG CACCGCAGGC AC | 111 | | | RNA. |
| GUGCCUGCGG UGCCAGAAAC CG | 112 | | | RNA |
| | | | | |
| GCAATGTAGC CGTATGTCCA TGC | 113 | HPV 18 primer | 2 | DNA |
| GCATGGACAT ACGGCTACAT TGC | 114 | | | DNA |
| GCAAUGUAGC CGUAUGUCCA UGC | 115 | | | RNA |
| GCAUGGACAU ACGGCUACAU UGC | 116 | | | RNA |
| | | | | |
| CACTTCACTG CAAGACATAG AAATAACCTG TGTATATT | 117 | HPV 18 HELPER | 3 | DNA |
| AATATACACA GGTTATTTCT ATGTCTTGCA GTGAAGTG | 118 | | | DNA |

| Sequence | Seq. ID No. | Type - HPV 16, - HPV 18, - Probe - Primer - Helper | Sub-Type 1, 2 or 3 | DNA or RNA |
|---|---|---|---|---|
| CACUUCACUG CAAGACAUAG AAAUAACCUG UGUAUAUU | 119 | | | RNA |
| AAUAUACACA GGUUAUUUCU AUGUCUUGCA GUGAAGUG | 120 | | | RNA |
| | | | | |
| TTATTAATAA GGTGCCTGCG GTGCCAGAAA CC | 121 | HPV 18 HELPER | 2 | DNA |
| GGTTTCTGGC ACCGCAGGCA CCTTATTAAT AA | 122 | | | DNA |
| UUAUUAAUAA GGUGCCUGCG GUGCCAGAAA CC | 123 | | | RNA |
| GGUUUCUGGC ACCGCAGGCA CCUUAUUAAU AA | 124 | | | RNA |
| | | | | |
| GACTCTGTGT ATGGAGACAC ATT | 125 | HPV 18 HELPER | 1 | DNA |
| AATGTGTCTC CATACACAGA GTC | 126 | | | DNA |
| GACUCUGUGU AUGGAGACAC AUU | 127 | | | RNA |
| AAUGUGUCUC CAUACACAGA GUC | 128 | | | RNA |
| | | | | |

Preferably, the target region comprises a sequence selected from the group consisting of those set forth in SEQ ID NOs: 9-12, 17-20, 29-32, 33-36, 45-48, and 73-76, or wherein said target region consists of a sequence present in a sequence selected from the group of consisting of those set forth in SEQ ID NOs: 5-8, 25-28, 57-60, 65-68, 77-80, and 81-84. Preferred oligonucleotides have, consist essentially of, consist of, or are substantially similar to the sequences set forth in SEQ ID NOs SEQ ID NOs: 5-12, 17-20, 25-36, 45-48, 57-60, 65-68 and 73-84.

The probes are isolated nucleic acids. The term "isolated nucleic acid" means an oligonucleotide or nucleic acid molecule which is present in a form not found in nature without human intervention (e.g., recombined with foreign nucleic acid, synthesized, isolated, or purified to some extent). Preferably an isolated nucleic acid is at least 75% homogenous. The probes may also contain additional nucleotides complementary to nucleic acid sequences contiguous to the target region and may also contain nucleotides not complementary to the targeted region, so long as such additional nucleotides do not prevent hybridization under stringent hybridization conditions. Non-complementary sequences, such as a promoter sequence, a binding site for RNA transcription, a restriction endonuclease recognition site, or sequences which will confer a desired secondary or tertiary structure such as a catalytic active site can be used to facilitate detection and/or amplification.

By "oligonucleotide," "nucleotide polymer" or "nucleic acid" is meant two or more nucleotide subunits covalently joined together. The sugar groups of the nucleotide subunits may be ribose, deoxyribose, or modified derivatives thereof such as 2'O-methyl ribose. The nucleotide bases may be modified by non-nucleotide moieties, that do not prevent preferential hybridization of the oligonucleotide to its complementary target nucleic acid. The nucleotide subunits may be joined by linkages such as phosphodiester linkages, modified linkages or by non-nucleotide moieties, that do not prevent preferential hybridization of the oligonucleotide to its complementary target nucleic acid. Modified linkages include those linkages in which a standard phosphodiester linkage is replaced with a different linkage, such as a phosphothionate linkage, or methylphosphonate linkage.

By "selective stringency hybridization conditions" is meant a set of parameters which allows the probes and target sequences of the invention to hybridize to one another forming a detectable probe:target duplex which can be used to distinguish HPV type 16 and or 18 from HPV types 6, 11, 31, 33, 35, 39, 45, 51, 52, or 58 or other HPV variants. A detailed description of these parameters is provided below in the "Description of the Preferred Embodiments" subsection IC. entitled, " Construction and Use of Hybridization Assay Probes." As but one example, the selective stringency hybridization conditions may preferably comprise 0.10M to 0.14M phosphate buffer containing approximately equimolar amounts of $Na_2HPO_4$ and $NaH_2PO_4$, approximately 1 mM EDTA, and 0.01 to 0.03% sodium dodecyl sulfate at 60 to 70°C.

In preferred embodiments the target region:

(a) comprises a sequence selected from the group consisting of those set forth in SEQ ID 9-12, 17-20, 29-32, and 33-36 or consists of a sequence selected from the group of consisting of those set forth in SEQ ID NOs: 5-8, and 25-28;
(b) comprises a sequence selected from the group consisting of those set forth in SEQ ID NOs: 45-48, and 73-76, or consists of a sequence selected from the group of consisting of those set forth in SEQ ID NOs: 57-60, 65-68, 77-80, and 81-84;
(c) is DNA or RNA;
(d) comprises a sequence selected from the group of consisting of those set forth in SEQ ID NOs: 33-36, and 45-48,
(e) comprises a sequence selected from the group consisting of those set forth in SEQ ID NOs: 9-12, 17-20, 29-32, and 73-76; and/or
(f) consists of a sequence selected from the group consisting of those set forth in SEQ ID NOs: 5-8, 25-28, 57-60, 65-68, 77-80, and 81-84.

In other especially preferred embodiments the probe preferentially hybridizes to nucleic acid of HPV Type 16 and/or Type 18 and not to HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, and/or 58 at 50 to 60°C in 0.04M to 0.06M lithium succinate buffer containing between 0.9 and 1.1% lithium lauryl sulfate, wherein said hybrid is stable for the detection of HPV Type 16 and/or Type 18 and not for the detection of HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, and/or 58.

The term "preferentially hybridize" is meant to indicate that under stringent hybridization assay conditions, hybridization assay probes can hybridize to their target nucleic acids to form stable probe:target hybrids which can be detected to indicate the presence of the target nucleic acid while the probes do not form a sufficient number of stable probe:non-target hybrids under these conditions to indicate the presence of a closely related non-target nucleic acid. Organisms "closely related" to HPV Types 16 and/or Type 18 include HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, or 58 (see, Van Ranst et al., J. Gen Vir., 73:2653-60, 1992).

Preferably the oligonucleotide comprises a sequence which is at least 90% complementary to said target sequence of 10 or more contiguous nucleotides, more preferably the oligonucleotide comprises a sequence which is 100% complementary to said target sequence of 10 or more contiguous nucleotides. In yet other preferred embodiments the oligonucleotide is 10 to 100 nucleotides in length, 14 to 50 bases in length, up to 40 nucleotides in length, 23-40 bases in length. The oligonucleotide may be linked to a second oligonucleotide sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

II. Nucleic Acid Hybrids

Another aspect of the present invention relates to compositions containing detectable nucleic acid hybrids made up of a hybridization assay probe and an HPV nucleic acid molecule having a nucleic acid sequence substantially complementary thereto. The hybrid is a stable nucleic acid structure comprising a double-stranded, hydrogen-bonded region, preferably 10 to 100 nucleotides in length. The term "hybrids" include RNA:RNA, RNA:DNA, or DNA:DNA duplex molecules. The hybridization probe present in the nucleic acid hybrid has one of the sequences noted above.

The term "substantially complementary" means that the nucleic acid sequence is able to preferentially hybridize under stringent hybridization assay conditions to a target nucleic acid region. Preferably, the probe has a region of at least 10 contiguous nucleotide bases which are complementary to the corresponding target region. More preferably,

the probe has a region of at least 14 contiguous nucleotide bases which are complementary to the corresponding target region.

The hybrid preferably is stable for the detection of HPV Type 16 and/or Type 18 and not for the detection of HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, and/or 58 and may further comprise a site for the initiation of nucleic acid synthesis. Under stringent hybridization conditions said oligonucleotide preferably hybridizes specifically to nucleic acid of HPV Type 16 and/or Type 18 and not to HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, or 58.

III. Helper Probes

In another aspect the invention features helper probes comprising an oligonucleotide, wherein said oligonucleotide comprises a sequence which will hybridize to a target sequence, wherein said target sequence has, consists essentially of, consists of, or is substantially similar to a sequence selected from the group consisting of SEQ ID NOs: 62, 64, 118, 120, 122, 124, 126, and 128.

In preferred embodiments, the oligonucleotide is substantially identical to (at least 70%) at least 10 contiguous nucleotides in a sequence selected from the group consisting of SEQ ID NOs: 61, 63, 117, 119, 121, 123, 125, and 127. In particular prefered embodiments the helper probes consist of these sequences.

It is also preferred that the oligonucleotide is at least 90% complementary to said subsequence of 10 or more contiguous nucleotides and more preferably is 100% complementary to said subsequence of 10 or more contiguous nucleotides. The oligonucleotide is preferably 10 to 100 nucleotides in length, 15 to 50 bases in length, up to 40 nucleotides in length, or 23-40 bases in length.

The preferred oligonucleotides have, consist essentially of, consist of, or are substantially similar to the sequences set forth in SEQ ID NOs: 61, 63, 117, 119, 121, 123, 125 and 127.

IV. Probe Mixes

Another aspect of the invention features probe mixes containing at least one hybridization probe and at least one helper probe for use in a hybridization assay. Helper probes can be used to facilitate hybridization of the probe:target duplex in a hybridization assay. Helper probes facilitate hybridization by enhancing the kinetics and/or the Tm of the target:hybridization probe duplex. Helper probes are described in Hogan and Milliman, U.S. Patent No. 5,030,557, which is incorporated by reference herein in its entirety, including any drawings.

Specifically, helper oligonucleotides are designed to bind to the target nucleic acid and impose a different secondary and tertiary structure on the target to facilitate binding of the assay probe to the target. The resulting hybrid of assay probe and target nucleic acid also exhibits a higher $T_m$ than the hybrid which results from addition of the probe in the absence of helper oligonucleotides. Because a substantial portion of this secondary and tertiary structure is not lost under conditions normally employed for nucleic acid hybridization, e.g., elevated temperature, presence of salt, presence of accelerators and the like, this residual structure can sterically inhibit, or even block, hybrid formation between a nucleotide multimer, for example a DNA or RNA oligomer being used as a probe, and its complementary sequence in the ribosomal RNA or other single-stranded nucleic acid such as mRNA or DNA which the probe targets. This inhibition can be reduced and even eliminated, by use of a "helper" oligonucleotide which binds to a portion of the RNA or DNA other than that being targeted by the probe, and which imposes new secondary and tertiary structure on the targeted region of the single-stranded nucleic acid whereby the rate of binding of the probe is accelerated. Thus, the rate of hybridization can be substantially increased and even permit hybridization to occur at a rate and under conditions otherwise adequate for an assay where, without the use of the helper, no substantial hybridization can occur.

In a preferred embodiment, nucleic acid hybridization assay probe component of the probe mix can detect the presence of HPV Type 16 and/or Type 18 and comprises a first oligonucleotide 10 to 100 bases in length having at least 14 out of 17 contiguous bases perfectly complementary to a first nucleic acid target region that consist of a sequence selected from the group consisting of those set forth in SEQ ID NOs: 9, 11, 17, 19, 29, 31, 33, 35, 45, 47, 73, and 75 or consists of a sequence selected from the group of consisting of those set forth in SEQ ID NOs: 5, 7, 25, 27, 57, 59, 65, 67, 77, 79, 81, and 83. The hybridization assay probe preferably distinguishes HPV Type 16 and/or Type 18 from HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, and/or 58 under selective hybridization conditions, i.e., under said conditions said hybridization assay probe hybridizes to HPV Type 16 and/or Type 18 RNA or DNA to form a detectable probe:target duplex, but does not hybridize to non-target nucleic acid from HPV 6, 11, 31, 33, 35, 39, 45, 51, 52, and/or 58 to form a detectable probe:non-target duplex.

Also in preferred embodiments the helper probe component of the probe mix comprises a second oligonucleotide which is at least 70% complementary to a second target sequence that comprises a sequence selected from the group consisting of those set forth in SEQ ID NOs: 61, 63, 121, 123, 125 and 127 or consists of a sequence selected from the group of consisting of those set forth in SEQ ID NOs: 117 and 119.

With respect to a hybridization assay probe or a helper probe, a "substantially similar" nucleotide sequence is a

nucleotide sequence identical to, or having no more than a 10% nucleotide base difference than an identified nucleotide sequence (excluding substitution of a RNA or DNA equivalent nucleotide, e.g., substituting T for U or U for T) and which enables a hybridization assay probe or helper probe to hybridize to HPV Type 16 and/or Type 18 nucleic acid under stringent hybridization conditions used to detect HPV Type 16 and/or Type 18 . With respect to amplification oligonucleotides, a "substantially similar" nucleotide sequence is a nucleotide sequence identical to, or having no more than a 20% nucleotide base difference than an identified nucleotide sequence (excluding substitution of a RNA or DNA equivalent nucleotide, e.g., substituting T for U or U for T) and which enables an amplification oligonucleotide to prime or initiate the amplification of HPV target nucleic acid under amplification conditions.

The phrases "consists essentially of" or "consisting essentially of" mean that the oligonucleotide has a nucleotide sequence substantially similar to a specified nucleotide sequence and is preferably no more than four additional nucleotides longer or two nucleotides shorter. Thus, these phrases contain both a sequence length limitation and a sequence variation limitation. Any additions, substitutions or deletions of an oligonucleotide consisting essentially of the specified nucleotide sequence do not deprive it of its basic and novel properties, vis, the ability to specifically hybridize with its target and function as a probe or a primer. For instance, with respect to hybridization and helper probes, any additions, substitutions or deletions would not prevent these probes from being able to preferentially hybridize under stringent hybridization assay conditions to its target nucleic acid over non-target nucleic acids. With respect to an amplification oligonucleotide, any additions, substitutions or deletions would not prevent it from being able to prime amplification reactions producing target HPV nucleic acid under amplification conditions.

V. Amplification Oligonucleotides

In another aspect, the invention features an amplification oligonucleotide for amplifying HPV Type 16 and/or Type 18 nucleic acid sequences. The oligonucleotide comprises a sequence of nucleic acids which has a region that is at least 70% complementary to a subsequence of 10 or more contiguous nucleic acids present in target sequence. The target sequence has, consists essentially of, consists of, or is substantially similar to a sequence selected from the group consisting of those set forth in SEQ ID NOs: 2, 4, 14, 16, 22, 24, 38, 40, 42, 44, 50, 52, 54, 56, 70, 72, 86, 88, 90, 92, 94, 96, 102, 104, 106, 108, 110, 112, 114, and 116.

In preferred embodiments, the oligonucleotide is DNA or RNA at least 70% identical to a subsequence of 10 or more contiguous nucleotides present in: (a) a sequence selected from the group of consisting of those set forth in SEQ ID NOs: 37, 39, 93, 95, 101, and 103; (b) a sequence selected from the group consisting of those set forth in SEQ ID NOs: 1, 3, 9, 11, 13, 15, 21, 23, 49, 51, 53, 55, 69, 71, 89, 91, 105, 107, 109, 111, 113, and 115; or (c) a sequence selected from the group consisting of those set forth in SEQ ID NOs: 41, 43, 85, and 87. The oligonucleotide is preferably at least 90% identical to said subsequence of 10 or more contiguous nucleotides, more preferably 100% identical to said subsequence of 10 or more contiguous nucleotides. The oligonucleotide is preferably 10 to 100 nucleotides in length, 15 to 50 bases in length, up to 40 nucleotides in length, or 23-40 bases in length.

In another aspect, the invention features amplification oligonucleotides useful for binding to, extending through, or transcribing HPV target regions. Located at the 5' end of the amplification oligonucleotide, which acts as a promoter sequence, is a sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase. Located at the 3' end of the same amplification oligonucleotide, is one or more sequences which acts as a target hybridizing region to HPV type 16 and or 18.

"RNA and DNA equivalent nucleotides" refer to RNA and DNA molecules having the equivalent base pair hybridization properties. RNA and DNA equivalents have different sugar groups (i.e., ribose versus deoxyribose), and may differ by the presence of uracil in RNA and thymine in DNA. The difference between RNA and DNA equivalents do not contribute to differences in substantially similar nucleic acid base sequences.

Amplification oligonucleotides are preferably 10 to 100 nucleotides in length, more preferably 22 to 44 nucleotides. Amplification oligonucleotides may have modifications, such as blocked 3' and/or 5' termini or additions including, but not limited to, specific nucleic acid sequences recognized by an RNA polymerase, (e.g., the promoter sequence for T7, T3, or SP6 RNA polymerase) sequences enhancing initiation or elongation of RNA transcription by an RNA polymerase (Kacian et al., U.S. Patent Number 5,399,491 incorporated herein in its entirety including drawings).

Amplification oligonucleotides can be used in nucleic acid amplification procedures, such as the polymerase chain reaction or transcription associated amplification reactions, such as that using RNA polymerase, and reverse transcriptase, as described by Kacian and Fultz supra. Other transcription based amplification systems are described in Sninsky et al., U.S. Patent No. 5,079,351. Both of these references are hereby incorporated by reference herein. Preferably, promoters which are recognized by an RNA polymerase such as T7, T3 or SP6 RNA polymerase are used for the transcription-based amplification.

The term "amplification" means increasing the number of nucleic acid molecules having at least one specific target nucleic acid sequence. In order to increase the amplification of oligonucleotides containing target sequences, applicants preferably employ amplification systems in which target-template strands containing a double-stranded promoter region

are produced to serve as templates for RNA polymerase. The target-template amplification is preferably carried out using a primer recognized by the DNA polymerase activity of reverse transcriptase.

VI. Methods Of Amplification and Detection

In another aspect the invention provides a method for selectively amplifying HPV Type 16 and/or Type 18 nucleic acid in a sample by amplifying the nucleic acid with one or more probes of the invention.

In yet another aspect the invention features a method for detecting HPV Type 16 and/or Type 18 in a sample potentially containing the HPV Type 16 and/or Type 18 comprising the steps of:

a) providing to said sample one or more nucleic acid hybridization assay probes of the invention; and
b) detecting the formation of said detectable probe:target duplex which is indicative of the presence of HPV Type 16 and/or Type 18.

In a preferred embodiment the target nucleic acid is amplified with an amplification probe and detected with a detection probe. Examples of most preferred combinations of a particular amplification probe with a particular detection probe (i.e., the best-mix combination) are shown in the examples presented herein.

In other aspects, methods are described for using the hybridization assay probes, helper probes and amplification oligonucleotides to detect HPV Type 16 and/or Type 18 and to distinguish HPV Type 16 and/or Type 18 from closely related organisms. These amplification assays involve amplifying target nucleic acid in a sample to be tested, contacting the amplified sequences under stringent hybridization assay conditions with a hybridization assay probe which preferentially hybridizes with HPV Type 16 and/or Type 18 nucleic acid over nucleic acids present in closely related organisms, and detecting or measuring the hybridized probe.

The sample is preferably a clinical sample such as sputum, urine, blood, uro-genital secretion, clinical swabs, tissue sections or nucleic acid isolated from a clinical sample. More preferably, the amplification assay will be used to detect HPV Type 16 and/or Type 18 directly from a clinical sample. Detection directly from a clinical sample means that culture of the sample is not required prior to carrying out the amplification assay.

Preferably the amplification assay utilizes a hybridization probe consisting of one those listed above. Helper probes for use in preferred embodiments of the amplification assay have, or are substantially similar to sequences selected from the group of Seq ID NOS: 117-128.

VII. Kits

The present invention also features a kit containing one or more of the hybridization assay probes or probe mixes of the invention. A kit contains all the necessary reagents to carry out the methods of detection described herein, for example one or amplification oligonucleotides or helper probes described herein. The kit may contain a one or more container means, a product insert label, and/or a buffer solution. Those skilled in the art will recognize that the probes of the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

The oligonucleotides targeted to HPV offer a rapid, objective and sensitive method of identification and quantitation of HPV by detecting the presence of specific nucleic acid sequences unique to different species and strains of HPV Type 16 and/or Type 18 . The probes of this invention can be used to identify, in hybridization assays, HPV from clinical samples. Combining an amplification step with a hybridization assay in the amplification assay increases the amount of target and thus the sensitivity of the assay. Both HPV type 16 and 18 can be amplified and detected in the same reaction vessel. A specially designed mismatch primer can amplify HPV Type 16 and/or Type 18 separately or simultaneously in the same reaction vessel. Probes can detect unspliced and heterogenous mRNA splices of HPV Type 16 and/or Type 18. Some of the probes of the present invention have been designed to exclude detection of mRNA targets which may be advantageous in certain applications.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

Following is description of HPV nucleic acids, methods of making and using oligonucleotide probes, and kits containing such probes. In particular, different types of oligonucleotide probes are described (including hybridization assay probes, helper oligonucleotides and amplification oligonucleotides) which are useful for detecting HPV Type 16 or Type 18 in a clinical sample, such as a vaginal swab, a cervical swab, a urethral swab, a tissue sample, a body fluid or an experimental solution.

Brief Description of the Drawings

Figures 1 and 2 are representations respectively of the E6 region in the HPV Type 16 and HPV Type 18 genome. The top line represents that portion of the genome between the designated base pairs (vertical lines). Subsequently lower lnes show splice acceptor sites and that portion of mRNA excised during transcription to produce either the E6* or E6** species of mRNA (area excised underlies triangle ;i.e., in Fig. 1 the area between bases 233-416 of the HPV type 18 RNA is excised during transcription resulting in the E6*mRNA). Still lower lines represent preferred probes, primers, and amplifying oligonucleotides (rectangles with SEQ ID NOS. adjacent) that were used in the Examples section below, i.e., which were used in amplifying and detecting either HPV type 16 or HPV type 18 E6, E6*, or E6** mRNA (specific example numbers located left of line).

I. Construction and Use of Hybridization Assay Probes.

A. Probe Design

Strands of deoxyribonucleic acid ("DNA") or ribonucleic acid ("RNA") are formed from nucleotide units joined in a specific arrangement, or sequence. Nucleotides each contain one "base" structure and are distinguished from one another by the base which they contain. Bases include adenine (A), cytosine (C), thymine (T), guanine (G), uracil (U), or inosine (I)) .

The structures of the bases in the nucleotides permit certain pairs of bases to interact with one another through the formation of hydrogen bonds. Generally, A is hydrogen bonded to T or U, while G is hydrogen bonded to C. At any point along the chain, therefore, one may find the classical base pairs AT or AU, TA or UA, GC, or CG. One may also find AG, GU and other "wobble" or mismatched base pairs. Bases which can hydrogen bond are said to be complementary to one another.

Two single strands of DNA or RNA may specifically align and associate ("hybridize") to form a double stranded structure in which the two strands are held together by the hydrogen bonds which form between pairs of complementary bases. When a first single-strand of nucleic acid contains sufficient contiguous complementary bases to a second, and those two strands are brought together under conditions promoting their hybridization, double-stranded nucleic acid results. Under appropriate conditions, double-stranded DNA/DNA, RNA/DNA, or RNA/RNA hybrids may be formed. Conditions which decrease the likelihood of forming a given double-stranded hybrid are said to be more stringent conditions than conditions in which hybrid formation is less likely.

A probe is generally a single-stranded nucleic acid having a base sequence which is complementary to some degree to a nucleic acid oligonucleotide "target region" comprising, consisting essentially of, or consisting of a "target sequence" sought to be detected. It may contain a detectable moiety such as a radioisotope, antigen or chemiluminescent moiety. A background description of the use of nucleic acid hybridization as a procedure for the detection of particular nucleic acids is described by Hogan et al., International Patent Application No. PCT/US87/03009, entitled "Nucleic Acid Probes for Detection and/Or Quantitation of Non-Viral Organisms," incorporated by reference herein in its entirety, including any drawings.

Using methods known to those skilled in the art, and described herein, regions of RNA or DNA sequences from HPV Type 16 and/or Type 18 were identified. Nucleic acids from different organisms and having different nucleotide sequences can be aligned in regions of homology based on a conserved primary sequence. Potential target sequences for the hybridization assay probes described herein were identified by noting variations in the homology of the aligned sequences.

The sequence evolution at each of the regions is mostly divergent. Because of this divergence, corresponding DNA regions of more distant phylogenetic relatives of HPV Type 16 and/or Type 18 show greater differences from HPV Type 16 and/or Type 18 RNA or DNA than do the DNAs of phylogenetically closer relatives. Sufficient variation between HPV Type 16 and/or Type 18 and its closest know phylogenetic relatives, HPV 6, 11, 31, 33, 35, 39, 45, 51, 52, or 58, was observed to allow identification of prospectivee target sites and to design hybridization assay probes useful for distinguishing between the nucleic acids of these organisms.

B. Oligonucleotide Probes

We have designed hybridization assay probes specific for HPV Type 16 and/or Type 18 , and we have successfully used those probes in a specific assay for the detection of HPV Type 16 and/or Type 18 , distinguishing the strains from each other and what are believed to be their most closely related taxonomic or phylogenetic neighbors. These probes have also been shown to function in an amplification assay for HPV Type 16 and/or Type 18. In addition, in a more preferred embodiment, we have used the probes in an amplification assay to detect HPV Type 16 and/or Type 18 directly from clinical samples such as vaginal swabs, sputum, biopsies, tissues, uro-genital fluid, uro-genital washes.

In addition, the probes can be used to detect HPV Type 16 and/or Type 18 in other clinical samples such as blood, and tissue sections, and in other samples such as swabs, secretions or biopsies. The featured probes preferably comprises, consists essentially of, or consist of one of the sequences identified above.

As illustrated by examples described below, the described hybridization assay probes can detect HPV Type 16 and/or Type 18 and distinguish it from HPV 6, 11, 31, 33, 35, 39, 45, 51, 52, or 58.

C. Hybridization

Hybridization assay probes and helper probes hybridize to their target sequence under stringent hybridization conditions. Oligonucleotides acting as helper probes or amplification oligonucleotides do not need to be able to preferentially hybridize to HPV Type 16 and/or Type 18 nucleic acid.

Preferential hybridization of hybridization assay probes to their target nucleic acids can be accomplished by choosing the appropriate hybridization assay conditions and proper probe design. The stability of the probe:target nucleic acid hybrid should be chosen to be compatible with the assay and washing conditions so that stable, detectable hybrids form only between nucleic acids having highly complementary sequences. Manipulation of one or more of the different assay parameters determines the exact sensitivity and specificity of a particular hybridization assay probe.

Preferential hybridization occurs under stringent hybridization assay conditions. In general, reducing the degree of complementarity of an oligonucleotide targeted region to its target sequence region decreases the degree or rate of hybridization of the probe oligonucleotide to its target sequence region.

Preferential hybridization can be measured using techniques known in the art and described herein, such as in the examples provided below. Preferably, there is at least a 100-fold difference between target and non-target hybridization signals, more preferably at least a 1,000-fold difference, even more preferably at least a 10,000-fold difference. Also preferably, non-target hybridization signals are not more than background level.

The following guidelines are useful for designing probes and determining specific stringent hybridization assay conditions. Because the sensitivity and specificity of hybridization reactions such as those described herein are affected by a number of factors, including the hybridization assay probe nucleotide sequence and length, the sequence of the target sequence region, the degree of homology between the target sequence and the analogous aligned HPV nucleic acid sequences from closely related organisms, the hybridization temperature, and the composition of hybridization reagents, the manipulation of one or more of those factors will determine the exact 0 sensitivity and specificity of a particular probe, whether perfectly complementary to its target or not. The importance and effect of various hybridization assay conditions, explained further herein, are known to those skilled in the art.

5 First, the stability of the probe:target nucleic acid hybrid should be chosen to be compatible with the assay conditions so that stable, detectable hybrids form only between nucleic acids having highly complementary sequences. Probes should be designed to have an appropriate melting temperature (Tm). This may be accomplished by varying the probe length and nucleotide composition (percentage of G + C versus A + T). The probe length and nucleotide composition are preferably chosen to correspond to a Tm about 2-10°C higher than the 5 temperature at which the final assay will be performed. For instance, the Tm can be increased by avoiding long A and T rich sequences, or by terminating the hybrids with G:C base pairs. The beginning and end points of the probe should be chosen so that the length and %G + C content result in a Tm about 2-10 °C higher than the temperature at which the final assay will be performed.

In general, the optimal hybridization temperature for an oligonucleotide is approximately 5°C below the melting temperature for a given duplex. Incubation at temperatures below the optimum temperature may allow mismatched base sequences to hybridize and can therefore decrease specificity. The longer the oligonucleotide, the more base pairs are present to hydrogen bond and, in general, the higher the Tm. The base composition of the probe is significant because G-C base pairs exhibit greater additional hydrogen bonding and therefore greater thermal stability than A-T base pairs. (See, e.g., 2 Sambrook, et al., Molecular Cloning: A Laboratory Manual 11 (2d ed. 1989) [hereinafter Molecular Cloning]) Thus, hybridization involving complementary nucleic acids of higher G-C content will be stable at higher temperatures.

To ensure specificity of a hybridization assay probe for its target, it is preferable to design probes which hybridize with target nucleic acids and not with non-target nucleic acids under conditions of high stringency. Under high stringency conditions only highly complementary nucleic acid hybrids will form. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between two nucleic acid strands in order to form a hybrid under those conditions. Stringency should be chosen to maximize the difference in stability between the probe: target hybrid and potential probe:non-target hybrids.

In addition, proper specificity may be achieved by minimizing the length of the hybridization assay probe having perfect complementarity to sequences of non-target organisms by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by constructing the probe to contain as many destabilizing mismatches to non-target sequences as possible. Whether a probe sequence

is appropriate for detecting only a specific type of organism depends largely on the thermal stability difference between probe:target hybrids and probe:non-target hybrids. In designing probes, the differences in these Tm values should be as large as possible (e. g., at least 2°C and preferably 5°C or more).

The length of the target nucleic acid sequence and, accordingly, the length of the probe sequence can also be important. In some cases, there may be several sequences from a particular region, varying in location and length, which will yield probes with the desired hybridization characteristics. In other cases, one sequence may be significantly better than another which differs from it merely by a single base. While it is possible for nucleic acids that are not perfectly complementary to specifically hybridize, the longest stretch of perfectly homologous base sequence generally determines hybrid stability.

Third, regions of RNA which are known to form strong internal structures inhibitory to hybridization are less preferred target regions. Likewise, probes with extensive self-complementarity should be avoided. If a strand is wholly or partially involved in an intramolecular or intermolecular hybrid it will be less able to participate in the formation of a new intermolecular probe:target hybrid. Ribosomal RNA molecules are known to form very stable intramolecular helices and secondary structures by hydrogen bonding. By designing a probe to a region of the target nucleic acid which remains substantially single-stranded under hybridization conditions, the rate and extent of hybridization between probe and target may be increased.

HPV target sequences may initially be present as part of a nucleic acid duplex. For example, a genomic DNA target occurs naturally in a double stranded form. The polymerase chain reaction (PCR) and transcription-based amplification systems can also give rise to a double stranded product. These double-stranded targets require denaturation prior to hybridization. Appropriate denaturation and hybridization conditions are known in the art (e.g., E.M. Southern, J. Mol. Biol. 98:503 (1975)).

The rate of hybridization may be measured by determining the $C_0T_{½}$. The rate at which a probe hybridizes to its target is a measure of the thermal stability of the target secondary structure in the probe region. The standard measurement of hybridization rate is the $C_0T_{½}$ which is measured as moles of nucleotide per liter times seconds. Thus, the $C_0T_{½}$ value is the concentration of probe times the half-life of hybridization at that concentration. This value is determined by hybridizing various amounts of probe to a constant amount of hybrid for a fixed time.

In one example, 0.05 pmol of target is incubated with 0.0012, 0.025, 0.05, 0.1 and 0.2 pmol of probe for 30 minutes. The amount of hybrid after 30 minutes is preferably measured by using Hybridization Protection Assay as described below. The signal is then plotted as the logarithmic unit of the percent of maximum Relative Light Units (RLU) (from the highest probe concentration) versus probe concentration (moles of nucleotide per liter). RLU are a measurement of the quantity of photons emitted by the labeled-probe measured by the luminometer. The $C_0T_{½}$ is found graphically from the concentration corre-sponding to 50% of maximum hybridization multiplied by the hybridization time in seconds. These values range from $9.0x10^{-6}$ to $9x10^{-5}$ with the preferred values being less than $3.5x10^{-5}$.

Other methods of nucleic acid reassociation can be used. For example, Kohne and Kacian, EP 229442, entitled "Accelerated Nucleic Acid Reassociation Method," describes a method to accelerate nucleic acid reassociation.

A preferred method to determine Tm measures hybridization using a hybridization protection assay (HPA) according to Arnold, et al., U.S. Patent No. 5,283,171, entitled "Homogeneous Protection Assay." Tm can be measured using HPA in the following manner. Probe molecules are labeled with an acridinium ester. Probe:target hybrids are formed in a lithium succinate buffer (0.1 M lithium succinate buffer, pH 5.0, 2 mM EDTA, 2 mM EGTA, 10% (w/v) lithium lauryl sulfate) using an excess amount of target. Aliquots of the solution containing the nucleic acid hybrids are then diluted in the lithium succinate buffered solution and incubated for five minutes at various temperatures starting below that of the anticipated Tm (typically 55°C) and increasing in 2-5° increments. This solution is then diluted with a mild alkaline borate buffer (0.15 M sodium tetraborate, pH 7.6, 5% (v/v) polyoxethylene ether (TRITON® X-100)) and incubated at a lower temperature (for example 50°C) for ten minutes.

Under these conditions the acridinium ester attached to the single-stranded probe is hydrolyzed, while the acridinium ester attached to hybridized probe is relatively protected from hydrolysis. Thus, the amount of acridinium ester remaining after hydrolysis treatment is proportional to the number of hybrid molecules. The remaining acridinium ester can be measured by monitoring the chemiluminescence produced from the remaining acridinium ester by adding hydrogen peroxide and alkali to the solution. Chemiluminescence can be measured in a luminometer (e.g., the Gen-Probe LEADER® I or LEADER ®50). The resulting data is plotted as percent of maximum signal (usually from the lowest temperature) versus temperature. The Tm is defined as the temperature at which 50% of the maximum signal remains. In addition to the method above, Tm may be determined by isotopic methods known to those skilled in the art (see e.g., Hogan et al., supra).

The Tm for a given hybrid varies depending on the nature of the hybridization solution used. Factors such as the concentration of salts, detergents, and other solutes can affect hybrid stability during thermal denaturation (see J. Sambrook, et al.,supra). Conditions such as ionic strength and incubation temperature under which a probe will be used should be taken into account in constructing a probe. It is known that the thermal stability of a hybrid nucleic acid increases with the ionic strength of the reaction mixture. On the other hand, the addition of chemical reagents which

disrupt hydrogen bonds, such as formamide, urea, DMSO and alcohols, can greatly reduce hybrid thermal stability and thereby increase the stringency of hybridization. In general, optimal hybridization for synthetic oligonucleotide probes of about 10-50 bases in length occurs approximately 5°C below the melting temperature for a given duplex. Incubation at temperatures below the optimum may allow mismatched base sequences to hybridize and can therefore result in reduced specificity.

Examples of specific stringent hybridization conditions for hybridization assay probes are provided in the examples described below. Additional sets of stringent hybridization conditions can be determined based on the present disclosure by those of ordinary skill in the art. (See e.g., Molecular Cloning, supra.)

D. Oligonucleotide Synthesis

Defined oligonucleotides may be produced by any of several well known methods, including automated solid-phase chemical synthesis using cyanoethylphosphoramidite precursors. Barone et al., Nucleic Acids Research12:4051 (1984). In addition, other well-known methods for construction of synthetic oligonucleotides may be employed. Molecular Cloning, supra,(2:11). Following synthesis and purification of an oligonucleotide, several different procedures may be utilized to determine the acceptability of the oligonucleotide in terms of size and purity. Such procedures include polyacrylamide gel electrophoresis and high pressure liquid chromatography, both of which are known to those skilled in the art. Once synthesized, selected oligonucleotide hybridization assay probes may also be labeled with a reporter group by an of several well known methods. Molecular Cloning, supra(2:11). Useful labels include radioisotopes as well as non-radioactive reporting groups. Isotopic labels include $^{3}H$, $^{35}S$, $^{32}P$, $^{125}I$, Cobalt and $^{14}C$. Isotopic labels can be introduced into an oligonucleotide by techniques known in the art such as nick translation, end labeling, second strand synthesis, reverse transcription, and by chemical methods. When using radio-labeled probes, hybridization can be detected by autoradiography, scintillation counting, or gamma counting. The chosen detection method depends on the hYbridization conditions and the particular radio-isotope used for labeling.

Non-isotopic materials can also be used for labeling, and may be introduced internally between nucleotides or at an end of the oligonucleotide. Modified nucleotides may be incorporated enzymatically or chemically. Chemical modifications of the probe may be performed during or after synthesis of the probe, for example, by the use of non-nucleotide linker groups as described by Arnold et al., entitled "Non-Nucleotide Linking Reagents for NUcleotide Probes," EPO application number 88308766.0, publication number 313219, incorporated by reference herein in its entirety including any drawings. Non-isotopic labels include fluorescent molecules, chemiluminescent molecules, enzymes, co-factors, enzyme substrates, haptens or other ligands.

Preferably, the hybridization assay probes are labeled with an acridinium ester. Acridinium ester labeling may be performed as described by Arnold et al., U.S. Patent No. 5,185,439 entitled "Acridinium Ester Labeling and Purification of Nucleotide Probes" issued February 9, 1993 and incorporated by reference herein in its entirety including any drawings.

II. Hybrids Containing a Hybridization Assay Probe and a HPV Target Sequence.

Another aspect of this invention is a hybrid formed by a hybridization assay probe and a target sequence from HPV Type 16 and/or Type 18 . The formed hybrid is useful for detecting the presence of the target. For example, acridinium ester ("AE") present in hybrid is resistant to hydrolysis in alkali solution while acridinium ester present in single-stranded nucleic acid is hydrolyzed in alkali solution. Thus, binding of AE-labeled probe to target can be detected, after hydrolysis of the unbound AE-labeled probe, by measuring chemiluminescence of acridinium ester remaining in the nucleic acid hybrid. Additionally, the formed hybrid can be used to as a basis to seperate hybridized target from unhybridized probe, thereby removing background due to unhybridized probe. For example, hybrid molecules can be selectively retained on hydroxyapatite columns or filters under conditions not permitting retention of single-stranded probe using methods well known to those skilled in the art (See, e.g., Sambrook, supra.).

III. Mixes of Hybridization Assay Probes and Helper Probes

Mixes of hybridization assay probes and helper probes can be used in the detection of HPV Type 16 and/or Type 18 . Helper probes are used to enhance the rate of nucleic acid hybridization of an assay probe with its target nucleic acid and to facilitate the hybridization of the hybridization assay probe to its target. In addition, helper probes are sufficiently complementary to their target nucleic acid sequence to form a helper probe:target duplex under stringent hybridization assay conditions. The stringent hybridization assay conditions used with a given helper probe are determined by the conditions in which a hybridization assay probe is used to preferentially hybridize to its target sequence.

Regions of single stranded RNA and DNA can be involved in secondary and tertiary structures even under stringent hybridization assay conditions. Such structures can sterically inhibit, or even block hybridization of a hybridization

assay probe to its target region. Hybridization of the helper probe alters the secondary and tertiary structure of the target nucleic acid, thereby rendering the hybridization assay probe target region more accessible. As a result helper probes enhance the kinetics and/or the Tm of the target:hybridization probe duplex. Helper probes are generally selected to hybridize to nucleic acid sequences located near the hybridization assay probe target region.

Helper probes which can be used with the hybridization assay probes of the present invention are targeted to nucleic acid sequence regions of the HPV genome and will preferablt contain at least 14 nucleotides of which at least 12 out of the 14 nucleotides are perfectly complementary to a nucleic acid sequence present in the HPV target region.

IV. Amplification Oligonucleotides and Amplification Assay Conditions

Methods of amplifying the number of target sequences in a sample can be combined with the use of probe sequences to increase the sensitivity of the detection assay. (Miller, et al., Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and PCR for Direct Detection of Mycobacterium tuberculosis in Clinical Specimens, J. Clin. Micro. 1994: 393-397; Reddy, et al., Mol. Cell. Probes 7: 121-126, 1993).

Amplification oligonucleotides can act as primers and may be part of promoter-primer combinations (i.e., a primer having an attached promoter sequence) to amplify a HPV Type 16 and/or Type 18 target sequence. Preferably the amplification oligonucleotide will have one of the following sequences: SEQ ID NOs: 1, 3, 13, 15, 21, 23, 37, 39, 41, 43, 49, 51, 53, 55, 69, 71, 89, 91, 93, 95, 101, 103, 105, 107, 109, 111, 113 and 115. In a more preferred embodiment, the amplification oligonucleotide will have one of the following sequences: SEQ ID Nos: 85, 87, 91, 93, 95, 101, 103, 105, 107, 109, 111, 113, and 115. In an even more preferred embodiment, the amplification oligonucleotide will have one of the following sequences: SEQ ID Nos: 109 and 111.

The degree of amplification observed with a set of primers or promoter-primers depends on several factors, including the ability of the oligonucleotides to hybridize to their specific target sequences and their ability to be extended or recognized by an RNA polymerase. While Oligonucleotides of different lengths and base composition may be used, more preferred amplification oligonucleotides have target binding regions of 30-60 bases and a predicted hybrid Tm of about 65°C.

A target nucleic acid sequence present on a nucleic acid molecule can be amplified using an amplification oligonucleotide 5' of the target sequence and an amplification oligonucleotide 3' of the target sequence. The preferred target sites for amplification oligonucleotides are regions greater than about 14 bases in length. The amplified region, defined by the amplification oligonucleotides, is preferably about 350 bases or less in length, and more preferably about 150 bases or less in length.

Parameters affecting probe hybridization such as Tm, complementarity and secondary structure also affect primer hybridization and therefore performance of the amplification oligonucleotides. These considerations, which were discussed above in the section concerning probe design, can be modified depending upon the amplification conditions. For example, amplification can be carried out under conditions of lower stringency than diagnostic hybridization assay conditions.

The degree of non-specific extension (primer-dimer or non-target copying) can affect amplification efficiency. Primers are preferably selected to have low self-or cross complementarity, particularly at the 3' ends of the sequence. Long homopolymer tracts and high GC content are preferably avoided to reduce spurious primer extension. Computer programs are commercially available to aid in this aspect of the design.

The terms "E6", "E6*" and "E6**" refer to the open reading frame of the HPV genome which encodes the number six "early" gene. This gene is designated by the prefix letter "E" for early, in conjunction with the arabic numeral "6" for the number six. The modifying terms designated by the characters "*" or "**" refer to alternatively spliced messanger RNAs which employ alternative acceptor splice sites within the E6 region of the HPV genome. Splicing at these sites during transcription of the E6 gene, results in the production of two different species of E6 mRNA's which differ in length from the canonical E6 mRNA. Therefore, three different E6 messenger RNA's may be generated depending upon which acceptor splice site is used within the E6 region of the genome. The HPV type 16 genome appears to express all three types of E6 mRNA while the HPV type 18 genome appears to express HPV type 18 E6 and E6* only.

In a particular embodiment of this invention primers and probes have been designed to amplify and detect each one of the heterogeneous E6 messenger RNA species. In a more preferred embodiment, two types of E6 messenger RNA may be amplified and detected by a set of primers and probes in the same reaction vessel. In the most preferred embodiment, all E6 messenger RNA species of a particular type may be simultaneously amplified and detected in the same reaction vessel by the primers and probes of this invention.

Examples

Described herein are preferred sequences for hybridization assay probes, helper probes, and amplification oligonucleotides designed to hybridize to target sequences in HPV Type 16 and/or Type 18 RNA or DNA. In addition,

preferred embodiments of mixes. of hybridization assay probes and helper probes useful for detecting HPV Type 16 and/or Type 18 are described. Also described are hybrids formed by a hybridization assay probe and a target sequence. Preferred methods for using the probes and amplification oligonucleotides to detect HPV Type 16 and/or Type 18 are included in this description.

The following examples illustrate several preferred embodiments of the present invention and should in no way be considered as limiting the scope of the invention which is defined in the appended claims. Various modifications of these examples could readily be performed by those skilled in the art without departing from the scope of the invention as defined in the claims.

Example 1: Probes Distinguishing HPV Type 16 from Type 18 and Vice Versa

This example illustrates the specificity of probes designed to be specific for HPV type 16 and 18. One hundred million copies of plasmid DNA containing a portion of the E6 gene from either HPV type 16 or type 18 were hybridized to a probe of SEQ ID NO. 5 or SEQ ID NO. 45.

To perform the hybridization, linearized plasmid contained HPV DNA sequences were heated to 95°C for 5 minutes in 50 µl of a solution containing $H_2O$ and cooled 1-2 minutes in a room temperature water bath. Then, 0.025-0.05 pmol (≅ 2.6 x $10^6$ RLUs/assay) of either (SEQ ID NO. 5) probe or SEQ ID NO. 45 probe was added to a final volume of 100 µl of 0.05 M lithium succinate pH 5, 0.6 M LiCl, 1% (w/v) lithium lauryl sulfate, 10 mM EDTA, 10 mM EGTA.

Hybridization was conducted at 60°C for 15 minutes. Following hybridiziation, 300 µl of 0.15 M sodium tetraborate pH 8.5, 1% TRITON X-100 at 60°C was added for 5 minutes. Samples were subsequently read in a luminometer equipped with automatic injection of 0.1% hydrogen peroxide in 1 mM nitric acid, followed by injection of a 1 N sodium hydroxide solution. The results, given in Table 1, are reported in Relative Light Units (RLU), a measure of the photons detected by the luminometer from labeled hybrids formed between probes SEQ ID NO. 5 and SEQ ID NO. 45 and their target nucleotide sequence.

Table 1.

| Specificity of Probes SEQ ID NOS. 5 & 45 for HPV Type 16 and 18. | | |
|---|---|---|
| | PROBE | |
| | SEQ ID NO. 5 | SEQ ID NO. 45 |
| TARGET | RLU | RLU |
| HPV type 16 E6 cloned DNA | 226,896 | 1,048 |
| HPV type 18 E6 cloned DNA | 745 | 817,751 |

The results (an average of two trial hybridization reactions) demonstrate that probe SEQ ID NO. 5 detects HPV type 16 E6 sequences in preference to those of HPV type 18. Similarly, the probe SEQ ID NO. 45 detects HPV type 18 E6 sequences in preference to those of HPV type 16.

This example illustrates that the designed oligonucleotides are capable of distinguishing target sequences from phylogenetically close species, since HPV type 16 and HPV type 18 are immediately related phylogenetically (see, Van Ranst et al., J. Gen. Vir., 73: 2653-60, 1992).

Example 2: Amplification and Detection of HPV Type 16 E6 DNA and RNA

This example illustrates the use of amplification oligonucleotides and hybridization assay probes targeted to HPV type 16 to facilitate amplification and detection of HPV type 16 nucleic acid. In this example, an assay probe for HPV type 16, of the same sense as E6 mRNA, was used to detect the products of a nucleic acid amplification method. (Kacian et al., supra.).

Cloned DNA representing a portion of HPV type 16 E6 DNA was purified using a standard mini-prep procedure with reagents purchased from Qiagen. Nucleic acid from cultured SiHa cells was prepared following trypsinization and centrifugation. The cell pellet was washed, resuspended and counted in phosphate buffered saline. Defined numbers of cells were pelleted and resuspended in Reagent I, a solution containing 3% (w/v) lithium lauryl sulfate, 30 mM sodium phosphate pH 6.8, 1.0 mM ethylene diamine tetra-acetic acid (EDTA), 1.0 mM ethylene glycol bis (beta-amino ethyl ether) N, N, N', N' tetra-acetic acid (EGTA) . Detergent was removed from samples by precipitation following addition of potassium acetate to a final concentration of 0.6 M. The nucleic acid contained in the supernatant was amplified directly.

The target nucleic acid was heated to 95°C for 5 minutes, cooled to 60°C for 15 minutes in 90 µl of a solution

containing 30 pmole of a promoter-primer synthesized with a promoter sequence 5' -AATTTAATACGACTCACTATAG-GGAGA-3' (SEQ ID NO. 97) at the 5' end and a target hybridizing region 5'-CAGGACACAGTGGCTTTTGAC-3' at the 3' end (SEQ ID NO. 85), and 30 pmole of a primer synthesized with the sequence 5'-GACATTATTGTTATAGTTTGTAT-GGAAC-3' (SEQ ID NO. 1) . Following a 5 minute incubation at 37°C, 900 U Moloney Murine Leukemia Virus (MMLV) reverse transcriptase and 400 U T7 RNA polymerase were added. The reactions were performed in 50 mM Tris-HCl, pH 7.6, 100 mM potassium acetate, 17.5 mM $MgCl_2$, 5.0 mM DTT, 2.0 mM spermidine, 6.2 mM rATP, 2.5 mM rCTP, 6.2 mM rGTP, 2.5 mM rUTP, 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 1 mM dTTP, 7 mM N-Acetyl-L-cysteine, 0.03 mM EDTA, 3% glycerol, and 10% Tween.

Following a three hour incubation at 37°C, 10 µl of each reaction was assayed by hybridization using an acridinium ester labeled probe synthesized with sequence 5'-GAACAGCAAT ACAACAAACC GTTGTGTG-3'(SEQ ID NO. 5) in 100 µl of 0.05 M lithium succinate pH 5, 0.6 M LiCl, 1% (w/v) lithium lauryl sulfate, 10 mM EDTA, 10 mM EGTA at 60°C for 15 minutes, followed by addition of 300 µl of 0.15 M sodium tetraborate pH 8.5, 1% TRITON X-100 at 60°C for 5 minutes.

Samples were read in a luminometer equipped with automatic injection of 0.1% hydrogen peroxide in 1 mM nitric acid, followed by injection of a 1 N sodium hydroxide solution. Results are given in Relative Light Units (RLU), a measure of the photons detected by the luminometer. The results shown are the average of two reactions.

Table 2 :

| Amplification and Detection of HPV Type 16 E6 DNA and RNA. | |
|---|---|
| HPV type 16 E6 nucleic acid | |
| copies cloned DNA | RLU |
| | |
| 0 | 945 |
| 1,000 | 5,247 |
| 10,000 | 35,620 |
| 1,000,000 | 881,886 |
| | |
| No. SiHa cells | |
| 100 | 1,335 |
| 1,000 | 2,182 |
| 10,000 | 428,602 |

These primers and probes were also able to amplify and detect DNA and RNA prepared from CaSki cells.

Example 3: Amplification and Detection of HPV Type 18 Sequences in HELA Cell Extracts

This example illustrates the use of amplification oligonucleotides and hybridization assay probes targeted to HPV type 18 to amplify and detect HPV type 18 nucleic acid. In this example, an assay probe designed to HPV type 18, of the same sense as E6 mRNA, was used to detect the products of target nucleic acid amplification.

Cloned DNA representing a portion of the HPV type 18 E6 DNA, or nucleic acid prepared from HeLa cells (which contain HPV type 18 E6 nucleic acid sequences), was amplified with a primer synthesized with SEQ ID NO. 53 and a promoter primer containing the sequence (SEQ ID NO. 97) 5'-AATTTAATACGACTCACTATAGGGAGA-3' at the 5' end and a target hybridizing sequence (SEQ ID NO. 109) at the 3' end.

Cultured HeLa cells were trypsinized and centrifuged, washed, resuspended and counted in phosphate buffered saline. Defined numbers of cells were pelleted and suspended in Reagent I. The detergent was precipitated with a 0.6 M final concentration of potassium acetate and the nucleic acid contained in the supernatant was amplified directly. Cloned DNA was placed into a mock specimen prepared by potassium acetate precipitation of detergent from Reagent I.

The target nucleic acid was heated to 95°C for 5 minutes, cooled to 60°C for 15 minutes, then cooled to 37°C in 90 µl of a solution containing 30 pmole each of the primer and promoter-primer. Following 5 minutes at 37°C, 900 U MMLV reverse transcriptase and 400 U T7 RNA polymerase were added. The reaction conditions were as described in Example 1.

Following a three hour incubation at 37°C, 10 µl of the reaction were assayed by hybridization using an 5 acridinium

ester labeled probe synthesized with SEQ ID NO. 45 in 100 µl of 0.05 M lithium succinate pH 5, 0.6 M LiCl, 1% (w/v) lithium lauryl sulfate, 10 mM EDTA, 10 mM EGTA at 55°C for 15 minutes, followed by addition of 300 µl of 0.15 M sodium tetraborate pH 8.5, 1% TRITON X-100 and incubation at 55°C for 5 minutes. Luminescence values of the samples were determined as described in Example 1.

Table 3 :

| Amplification and Detection of HPV Type 18 Sequences in HELA Cell Extracts. | |
|---|---|
| Copies cloned HPV type 18 E6 DNA | RLU |
| 0 | 862 |
| 100 | 15,993 |
| 1,000 | 279,565 |
| 10,000 | 820,591 |
| | |
| No. HeLa Cells | |
| 10,000 | 1,216,105 |
| 1,000 | 380,040 |
| 100 | 40,085 |

* Plasmid DNA was added following detergent precipitation. One HeLa cell contains approximately 10-50 copies of DNA. Therefore, 100 cells HeLa are equivalent to approximately 1,000-5,000 copies of DNA. HeLa number values which are lower than predicted from cloned DNA, are most likely the result of a loss of material during detergent precipitation.

Example 4: Amplification and Detection of Both HPV Type 16 and Type 18 in the Same Reaction Vessel

Amplification oligonucleotides, as described herein, were designed to amplify both HPV type 16 and 18 DNA in the same reaction vessel, followed by detection with specific probes which can distinguish between the 16 and 18 variants. In this example, cloned target DNA from both HPV type 16 and 18 was amplified, followed by detection with the assay probes described.

Cloned DNA representing the HPV type 16 E6 DNA was amplified with a non-T7 primer consisting of SEQ ID NO. 1 and a promoter primer synthesized with a 3' target hybridizing sequence, SEQ ID NO. 85. Cloned DNA representing HPV type 18 DNA was amplified with a primer consisting of SEQ ID NO. 53 and a promoter primer with a 3' target hybridizing sequence SEQ ID NO. 109, both promoter primers containing the sequence 5'-AATTTAATACGACTCAC-TATAGGGAGA-3' at their 5' end.

The target nucleic acid in detergent precipitated Reagent I was heated to 95°C for 5 minutes, cooled to 60°C for 15 minutes, then cooled to 37°C in 90 µl of a solution containing 30 picomoles of each primer and promoter primer. Following 5 minutes at 37°C, 900 U MMLV reverse transcriptase and 400 U T7 RNA polymerase were added. The reaction conditions are as described in Example 1.

Following a three hour incubation at 37°C, ten µl of the reaction was assayed by hybridization using an acridinium ester labeled probe synthesized SEQ ID NO. 45, in 100 µl of 0.05 M lithium succinate pH 5, 0.6 M LiCl, 1% (w/v) lithium lauryl sulfate, 10 mM EDTA, 10 mM EGTA at 55°C for 15 minutes, followed by addition of 300 µl of 0.15 M sodium tetraborate pH 8.5, 1% TRITON X-100 and incubation at 55°C for 5 minutes. Ten µl of each reaction was also analyzed with acridinium ester labeled probe SEQ ID NO. 5 in 100 µl as described for probe SEQ ID NO. 45 except that the incubations were performed at 60°C. Luminescence values of the samples were determined as described in Example 1.

As can be determined from Table 4, the designed oligonucleotide primers work well in the same reaction vessel. The amplification of either HPV Type 16 and/or Type 18 does not hinder amplification of the other Papillomavirus and the presence of either HPV Type 16 and/or Type 18 hybridization probe does not interfere with the ability of the other probe to distinguish HPV type 16 from 18 or vice versa. In fact, the specificity of each probe is ten times greater for its target than for a non-target nucleotide sequence.

Table 4 :

| Co-Amplification of HPV Type 16 and 18. | | | |
|---|---|---|---|
| | PROBE | RLU | |
| | | SEQ ID NO. 5 | SEQ ID NO. 45 |
| Copies HPV type 16 DNA | Copies HPV type 18 DNA | | |
| 1,000,000 | 1,000,000 | 1,115,026 | 1,057,903 |
| 10,000 | 1,000,000 | 643,415 | 974,109 |
| 1,000 | 1,000,000 | 152,810 | 944,290 |
| 100 | 1,000,000 | 6,852 | 930,188 |
| 0 | 0 | 1,243 | 1,140 |
| 1,000,000 | 1,000,000 | 991,682 | 886,852 |
| 1,000,000 | 10,000 | 1,003,151 | 821,532 |
| 1,000,000 | 1,000 | 1,001,966 | 389,796 |
| 1,000,000 | 100 | 997,714 | 10,157 |

Example 5: Designed Mismatch Primer Can Amplify HPV Type 16 and/or Type 18 Separately or Simultaneously

A promoter primer (SEQ ID NO. 113) was designed with mismatches to both HPV type 16 and 18 target nucleotide sequences. This promoter primer design has the ability to amplify both HPV type 16 and 18 DNA target sequences separately, or concurrently in the same reaction vessel.

Double stranded cloned targets were amplified with 30 pmole each of promoter primer synthesized with promoter SEQ ID NO. 97 at the 5' end and a target hybridizing SEQ ID NO. 113 or SEQ ID NO. 93 at the 3' end, in the presence of a primer synthesized with SEQ ID NO. 69 for HPV type 18 or a primer synthesized with SEQ ID NO. 37 for HPV type 16.

A 75 µl volume of the target nucleic acid was heated to 95°C for 15 minutes and cooled to 42°C. Following 5 minutes at 42°C, 600 U MMLV reverse transcriptase and 300 U T7 RNA polymerase were added. Amplification reactions were performed in 50 mM Tris-HCl, pH 8.5, 5 mM potassium chloride, 20 mM $MgCl_2$, 4 mM rATP, 4 mM rCTP, 4 mM rGTP, 4 mM rUTP, 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 1 mM dTTP, 20 mM N-Acetyl-L-cysteine, and 5% glycerol.

Following a two hour incubation at 42°C, triplicate amplification reactions were pooled and 100 µl of each pool was hybridized with an acridinium ester labeled probe synthesized with SEQ ID NO. 73 for the detection of HPV type 18 or SEQ ID NO. 33 for the detection of HPV type 16.

Table 5 :

| Co-Amplification of HPV Type 16 and 18 Using a Mismatch Promoter Primer | | | |
|---|---|---|---|
| | RLU | | |
| Primers: | SEQ ID NO<br>No. 93/37<br>T7/Non-T7 | SEQ ID<br>No.113/69<br>T7/Non-T7 | SEQ ID<br>Nos. 113/37or 69<br>T7/Non-T7 |
| Probe: | SEQ ID<br>No. 33 | SEQ ID<br>No. 73 | SEQ ID<br>Nos. 33 and 73 |
| Target | | | |
| 1,000 copies HPV type 16 DNA | 251,581 | 6,495 | 158,771 |
| 1,000 copies HPV type 18 DNA | 2,335 | 255,227 | 528,971 |
| 0 copies | 4,509 | 3,379 | 6,105 |

As can be seen from an examination of Table 5, the single mismatch promoter primer of SEQ ID NO. 113, can amplify two HPV nucleic acids (16 & 18) differing in sequence by 3 bases in the primer binding site.

Example 6: Amplification and Detection of Spliced E6* mRNA of HPV Type 16

This example illustrates the use of amplification oligonucleotides and hybridization assay probes for HPV type 16 to amplify and specifically detect E6* mRNA of HPV type 16. In this example, an assay probe of the same sense as the target E6* RNA nucleic acid was used to detect the products of a target nucleic acid amplification method.

Cultured SiHa cells were trypsinized, centrifuged, washed, resuspended and counted in phosphate buffered saline. Defined numbers of cells were pelleted and resuspended in Reagent I (i.e., Example 1). Detergent was removed from samples suspended in Reagent I by precipitation following addition of potassium acetate to a final concentration of 0.6 M. The nucleic acid contained in the supernatant was amplified directly.

The target nucleic acid was heated to 95°C for 5 minutes, cooled to 60°C for 15 minutes in 90 µl of a solution containing 30 pmole of one of two promoter-primers synthesized with a T7 promoter at the 5' end. The first promoter-primer had a target hybridizing region at the 3' end (SEQ ID NO. 89), and the second promoter-primer had a a target hybridizing region (SEQ ID NO. 21) at its 3' end. Following 5 minutes at 37°C, 900 U MMLV reverse transcriptase and 400 U T7 RNA polymerase were added. The reaction conditions were as described in Example 1. [performed in 50 mM Tris-HCl, pH 7.6, 100 mM potassium acetate, 17.5 mM $MgCl_2$, 5.0 mM DTT, 2.0 mM Spermidine, 6.2 mM rATP, 2.5 mM rCTP, 6.2 mM rGTP, 2.5 mM rUTP, 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 1 mM dTTP, 7 mM N-Acetyl-L-cysteine, 0.03 mM EDTA, 3% glycerol, Tween-20®]. Following a three hour incubation at 37°C, 10 µl of the reaction was assayed by hybridization using an acridinium ester labeled probe synthesized with SEQ ID NO. 9 in 100 µl of 0.05 M lithium succinate pH 5, 0.6 M LiCl, 1% (w/v) lithium lauryl sulfate, 10 mM EDTA, 10 mM EGTA at 60°C for 15 minutes, followed by addition of 300 µl of 0.15 M sodium tetraborate pH 8.5, 1% TRITON X-100 and incubation at 60°C for 5 minutes. Luminescence values of the samples were determined as described in Example 1. The results are the average of two reactions.

Table 6 :

| Amplification and Detection of HPV Type 16 E6* RNA. | |
|---|---|
| No. SiHa cells | RLU |
| 0 | 656 |
| 1,000 | 18,391 |
| 10,000 | 418,717 |

These primers and probes were also able to amplify and detect DNA and RNA prepared from CaSki cells.

Example 7: Hybridization Assay Probes Detect HPV Type 16 E6 E6* and E6** Nucleic Acid

Assay probes designed for HPV type 16 are able to detect products of the amplification of E6 nucleic acid, including un-spliced, E6, E6* and E6** mRNA targets. The primer and promoter primer were oriented in such a way that the predominant amplification product was the same sense as the messenger RNA. In this example, nucleic acid prepared from Caski cells was used. Defined numbers of cells were pelleted and suspended in Reagent I. Potassium acetate was added to a final concentration of 0.6 M to precipitate the detergent and the nucleic acid contained in the supernatant was amplified directly. Nucleic acid from $1.6 \times 10^4$ cells was amplified as described in Example 1 with a promoter primer synthesized with a 5' T7 promoter and a 3' target hybridizing SEQ ID NO. 21, and 30 pmole of a primer synthesized with SEQ ID NO. 13. Ten µl of each reaction was hybridized to probes synthesized with SEQ ID NO. 29, SEQ ID NO. 25, or SEQ ID NO. 17.

Table 7 :

| Specific Detection of Un-Spliced and Spliced E6 MRNA Prepared From CASKI Cells. | | | |
|---|---|---|---|
| | RLU | | |
| Target : HPV16 | E6 mRNA | E6* mRNA | E6** mRNA |
| Probe : | SEQ ID NO. 29 | SEQ ID NO. 25 | SEQ ID NO. 17 |

Table 7 : (continued)

| Specific Detection of Un-Spliced and Spliced E6 MRNA Prepared From CASKI Cells. | | | |
|---|---|---|---|
| | RLU | | |
| Sample | | | |
| 16,000 cells | 551,715 | 38,908 | 236,324 |
| 0 cells | 1,006 | 1,295 | 2,375 |

The results indicated in Table 7 support reports in the literature that the heterogeneous splice variations of E6 mRNA vary in quantity within the cell.

Example 8: Primers and Probes Detect HPV Type 18 E6* MRNA

Primers and probes capable of detecting the E6* mRN an HPV type 18 were designed. Defined numbers of HeLa cells were suspended in Reagent I and the nucleic acid was recovered following detergent precipitation, as in Example 7, and amplified directly. Amplification reactions were performed with a promoter primer synthesized with a 5' promoter SEQ ID NO. 97 and a target hybridizing region SEQ ID NO. 101, and a primer consisting of SEQ ID NO. 49. Reaction conditions were as in Example 1. Ten µl of the amplification reaction was hybridized with an acridinium ester labeled probe synthesized with SEQ ID NO. 77 in 100 µl of 0.05 M lithium succinate pH 5, 0.6 M Licl, 1% (w/v) lithium lauryl sulfate, 10 mM EDTA, 10 mM EGTA at 55°C for 15 minutes, followed by addition of 300 µl of 0.15 M sodium tetraborate pH 8.5, 1% TRITON X-100 at 55°C for 5 minutes. Luminescence values were determined as in Example 1.

Table 8 :

| Amplification and Detection of HPV Type 18 E6* mRNA | |
|---|---|
| HPV type 18 E6* | |
| No. HeLa cells | RLU |
| 10,000 | 1,309,947 |
| 1,000 | 18,231 |
| 100 | 2,750 |
| 0 | 1,144 |

Example 9: Probes & Primers Amplify & Detect Both Spliced and Un-Spliced HPV Type 18 E6 mRNA

Primers and probes were designed to amplify and detect un-spliced and spliced E6 sequences of HPV type 18. A primer synthesized with SEQ ID NO. 41 and a promoter primer synthesized with a 5' promoter sequence 5'--AATT-TAATACGACTCACTATAGGGAGA-3' and a 3' target hybridizing region SEQ ID NO. 109 were used to amplify cloned DNA representing HPV type 18 E6 sequences. The target nucleic acid was heated to 95°C for 15 minutes, and cooled to 42°C in 75 µl of a solution containing 25 picomoles of the primer and promoter primer. Following 5 minutes at 42°C, 600 U MMLV reverse transcriptase and 300 U T7 RNA polymerase were added. Amplification reactions were performed in 50 mM Tris-HCl, pH 8.5, 35 mM potassium acetate, 20 mM $Mgcl_2$, 4 mM rATP, 4 mM rCTP, 4 mM rGTP, 4 mM rUTP, 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 1 mM dTTP, 20 mM N-Acetyl-L-cysteine, and 5% glycerol. Following a two hour incubation at 42°C, 20 µl of each reaction was assayed by hybridization using an acridinium ester labeled probe of SEQ ID NO. 81 for E6* detection, SEQ ID NO. 65 for E6 detection, or SEQ ID NO. 57 for both E6 and E6* detection, using conditions described in Example 1. An unlabeled helper probe consisting of sequence SEQ ID NO. 61 was used with probes 81 and 65, an unlabeled helper probe of sequence SEQ ID NO. 117 was used with probe SEQ ID NO. 57.

Table 9

| Amplification and Detection of HPV Type 18 Spliced and Un-Spliced E6 mRNA | | | |
|---|---|---|---|
| | RLU | | |
| Target : HPV18 | E6* | E6 | E6 and E6* |
| Probe : | SEQ ID NO. 81 | SEQ ID NO. 65 | SEQ ID NO. 15 |

Table 9 (continued)

| Amplification and Detection of HPV Type 18 Spliced and Un-Spliced E6 mRNA | | | |
|---|---|---|---|
| | RLU | | |
| Helper : | SEQ ID NO. 61 | SEQ ID NO. 61 | SEQ ID NO. 117 |
| E6 Target added to reaction : | | | |
| $10^6$ un-spliced | 1,057 | 912,867 | 975,288 |
| $10^5$ un-spliced | 703 | 343,900 | 406,958 |
| $10^4$ un-spliced | 546 | 208,246 | 255,093 |
| $10^3$ un-spliced | 582 | 58,946 | 90,836 |
| $10^6$ spliced | 1,387,098 | 5,709 | 1,499,335 |
| $10^5$ spliced | 704,412 | 3,430 | 807,331 |
| $10^4$ spliced | 184,847 | 1,446 | 300,340 |
| $10^3$ spliced | 27,907 | 936 | 46,798 |
| Negative | 564 | 1,542 | 619 |

Example 10: HPV Type 16 & 18 Detection from Clinical Samples

Endocervial swabs from patients attending a clinic were placed into a tube containing 5 ml of Reagent I. The swabs were expressed and discarded. Nucleic acid from Reagent I was extracted following addition of potassium acetate to 0.6 M and removal of the detergent pellet by centrifugation. A sample of the nucleic acid in the supernatant was analyzed for the presence of HPV by established procedures. In one test, a portion of the nucleic acid was phenol chloroform extracted and amplified by polymerase chain reaction using published primers targeting the L1 gene sequences. Samples were also assayed for amplification of L1 sequences by agarose gel analysis directly or following restriction endonuclease digestion. Alternatively, samples were analyzed by hybridization with acridinium ester labeled probes directed to published sequences of HPV type 16 and 18. Samples positive by gel for types other than HPV type 16 or HPV type 18 were assayed by with acridinium ester labeled L1 probes corresponding to the identified type (HPV 6, 11, 31, 33, 35, 39, 45, 51, 52 and 58) for confirmation. Samples characterized in this manner were then tested in the amplification format using the primers and promoter primers described herein. Amplified samples were assayed by HPA with detection probes directed to HPV type 16 (SEQ ID NO. 5) or HPV type 18 (SEQ ID NO. 45) sequences.

Table 10: Detection of HPV Type 16 & 18 From Clinical Samples

| Clinical isolate number | HPV type by L1 PCR RFLP | HPV type by AE probe hybridization | Amplification result |
|---|---|---|---|
| | | | |
| 16 | 6 | 6 | 16- |
| 25 | 6 | 6 | 16- |
| 28 | 6 | 6 | 16- |
| 797 | 6 | 6+11+33 | 16- |
| 3 | 16 | 16 | 16+ |
| 29 | 16 | 16 | 16+ |
| 41 | 16 | 16 | 16+ |
| 72 | 16+6 | 16+6 | 16+ |
| 133 | 16 | 16 | 16+ |
| 146 | 16 | 16 | 16+ |
| 173 | 16+33 | 16+33 | 16+ |
| 185 | 16+Gx3 (*) | 16 | 16+ |
| 190 | 16 | 16 | 16+ |
| 204 | 16 | 16 | 16+ |
| 216 | 16 | 16 | 16+ |
| 224 | 16+Gx3 (*) | 16 | 16+ |
| 234 | 16 | 16 | 16+ |
| 293 | 16+35 | 16+35 | 16+ |
| 341 | 16 | 16 | 16+ |
| 364 | 16 | 16 | 16+ |
| 368 | 16+58 | 16 | 16+ |
| 369 | 16+58 | 16 | 16+ |
| 370 | 16+58 | 16 | 16+ |
| 789 | 16 | 16 | 16+ |
| 796 | 16 | 16 | 16+ |
| 96 | 18 | 18 | 18+ |

| | | | |
|---|---|---|---|
| 114 | 18 | 18 | 18+ |
| 177 | 18+Gx9 (*) | 18 | 16-18+ |
| 290 | 18 | 18+16 | 18 |
| 297 | 18 | 18 | 18 |
| 798 | 11+18 | 18 | 18 |
| 101 | 31 | 31 | 16- |
| 118 | 31 | 31 | 16- |
| 115 | 33 | 33 | 16- |
| 166 | 33 | 33 | 16- |
| 791 | 33 | 33 | 16- |
| 192 | 39 | N.D. | 16- |
| 109 | 58 | N.D. | 16- |
| 175 | 58 | N.D. | 16- |
| 176 | 58 | N.D. | 16- |
| 182 | 58 | N.D. | 16- |
| 779 | 58 | N.D. | 16- |

The data in this table are reported as positive if the RLU value was over the value of the negative control by a factor of 1.5-2.0 X for the luminometer employed in this example. Acridinium ester labeled probe SEQ ID NO. 5 was used for detection of HPV type 16 and acridinium labeled probe SEQ ID NO. 45 was used with unlabeled helper probe SEQ ID NOs. 121, 123, 127, or 125 for detection of HPV type 18.

The data shown in the various examples described above confirm that the novel probes herein described and claimed are capable of distinguishing HPV from their known nearest phylogenetic neighbors. Furthermore, complementary oligonucleotide probes, i.e., those having the same sense as the target, are utilized to detect the products of target amplification procedures now being utilized to increase the detection sensitivity of assays for organisms.

Sequence information was obtained experimentally and from published information. (See Weisburg, et al., J. Bacteriol 171:6455 (1989).) Experimental information was obtained by isolating and sequencing RNA or DNA from various organisms using standard techniques known in the art. More specifically, RNA sequence information was obtained by first using oligonucleotide primers complementary to conserved regions which vary little between prokaryotic organisms. The oligonucleotide primers were hybridized to the conserved regions in purified RNA and extended with the enzyme reverse transcriptase and deoxyribonucleotides to produce cDNA. E.g., Lane et al., Proc. Nat'l Acad. Sci. USA 82:6955 (1985).

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

Those references not previously incorporated herein by reference, including both patent and non-patent references, are expressly incorporated herein by reference for all purposes. Other embodiments are within the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: GEN-PROBE INCORPORATED

(B) STREET: 9880 CAMPUS POINT DRIVE

(C) CITY: SAN DIEGO

(D) STATE: CALIFORNIA

(E) COUNTRY: UNITED STATES OF AMERICA

(F) POST CODE: 92121

(ii) TITLE OF INVENTION: NUCLEIC ACID PROBES COMPLEMENTARY TO HUMAN PAPILLOMAVIRUS NUCLEIC ACID AND RELATED METHODS AND KITS

(iii) NUMBER OF SEQUENCES: 128

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: MOON, DONALD KEITH
BREWER & SON

(B) STREET: QUALITY HOUSE
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(A) TELEPHONE: (0171) 242-2047

(B) TELEFAX: (0171) 831-0298

(C) TELEX:

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb storage

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: IBM P.C. DOS 6.0

(D) SOFTWARE: Word Perfect 6.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: NOT YET ASSIGNED

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
Prior applications total, including application described below: ONE
(A) APPLICATION NUMBER: 60/006,854
(B) FILING DATE: 15-NOV-1995

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
GACATTATTG TTATAGTTTG TATGGAAC 28

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
GTTCCATACA AACTATAACA ATAATGTC 28

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
GACAUUAUUG UUAUAGUUUG UAUGGAAC 28

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GUUCCAUACA AACUAUAACA AUAAUGUC 28

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAACAGCAAT ACAACAAACC GTTGTGTG 28

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CACACAACGG TTTGTTGTAT TGCTGTTC 28

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAACAGCAAU ACAACAAACC GUUGUGUG 28

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CACACAACGG UUUGUUGUAU UGCUGUUC 28

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
GACGTGAGGT GTATTAACTG TCAAAAG 27

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
CTTTTGACAG TTAATACACC TCACGTC 27

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
GACGUGAGGU GUAUUAACUG UCAAAAG 27

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
CUUUUGACAG UUAAUACACC UCACGUC 27

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CCATGCATGA TTACAGCTGG GTTTCTC 27

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAGAAACCCA GCTGTAATCA TGCATGG 27

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCAUGCAUGA UUACAGCUGG GUUUCUC 27

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GAGAAACCCA GCUGUAAUCA UGCAUGG 27

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TACGTGTTCT TGATGATCTC ACGTCG 26

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGACGTGAGA TCATCAAGAA CACGTA 26

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

UACGUGUUCU UGAUGAUCUC ACGUCG 26

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGACGUGAGA UCAUCAAGAA CACGUA 26

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GTGTGTACTG CAAGCAACAG TTACTG 26

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
CAGTAACTGT TGCTTGCAGT ACACAC 26

(2) INFORMATION FOR SEQ ID NO:23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
GUGUGUACUG CAAGCAACAG UUACUG 26

(2) INFORMATION FOR SEQ ID NO:24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
CAGUAACUGU UGCUUGCAGU ACACAC 26

(2) INFORMATION FOR SEQ ID NO:25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
CTTTTGACAG TTAATACACC TCACG 25

(2) INFORMATION FOR SEQ ID NO:26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGTGAGGTGT ATTAACTGTC AAAAG 25

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CUUUUGACAG UUAAUACACC UCACG 25

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CGUGAGGUGU AUUAACUGUC AAAAG 25

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AAAGTCATAT ACCTCACGTC GC 22

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCGACGTGAG GTATATGACT TT 22

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAAGUCAUAU ACCUCACGUC GC 22

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GCGACGUGAG GUAUAUGACU UU 22

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GAAACCCAGC TGTAATCATG C 21

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GCATGATTAC AGCTGGGTTT C 21

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:
GAAACCCAGC UGUAAUCAUG C 21

(2) INFORMATION FOR SEQ ID NO:36:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:
GCAUGAUUAC AGCUGGGUUU C 21

(2) INFORMATION FOR SEQ ID NO:37:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:
GATCATCAAG AACACGTAG 19

(2) INFORMATION FOR SEQ ID NO:38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:
CTACGTGTTC TTGATGATC 19

(2) INFORMATION FOR SEQ ID NO:39:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GAUCAUCAAG AACACGUAG 19

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CUACGUGUUC UUGAUGAUC 19

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GGAACTGAAC ACTTCACTGC AAGACATAGA AATAACC 37

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GGTTATTTCT ATGTCTTGCA GTGAAGTGTT CAGTTCC 37

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GGAACUGAAC ACUUCACUGC AAGACAUAGA AAUAACC 37

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:
GGUUAUUUCU AUGUCUUGCA GUGAAGUGUU CAGUUCC 37

(2) INFORMATION FOR SEQ ID NO:45:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:
GGAAAAACTA ACTAACACTG GGTTATACAA T 31

(2) INFORMATION FOR SEQ ID NO:46:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:
ATTGTATAAC CCAGTGTTAG TTAGTTTTTC C 31

(2) INFORMATION FOR SEQ ID NO:47:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:
GGAAAAACUA ACUAACACUG GGUUAUACCA 31

(2) INFORMATION FOR SEQ ID NO:48:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

AUUGUAUAAC CCAGUGUUAG UUAGUUUUUC C 31

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CATAGAAATA ACCTGTGTAT ATTGCAAG 28

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CTTGCAATAT ACACAGGTTA TTTCTATG 28

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CAUAGAAAUA ACCUGUGUAU AUUGCAAG 28

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

CUUGCAAUAU ACACAGGUUA UUUCUAUG 28

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GACATTATTC AGACTCTGTG TATGGAG 27

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CTCCATACAC AGAGTCTGAA TAATGTC 27

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GACAUUAUUC AGACUCUGUG UAUGGAG 27

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CUCCAUACAC AGAGUCUGAA UAAUGUC 27

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:
GCAAGACAGT ATTGGAACTT ACAGAG 26

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:
CTCTGTAAGT TCCAATACTG TCTTGC 26

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:
GCAAGACAGU AUUGGAACUU ACAGAG 26

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:
CUCTGUAAGU UCCAAUACUG UCUUGC 26

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

CCTGTGTATA TTGCAAGACA GTATTG 26

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

CAATACTGTC TTGCAATATA CACAGG 26

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

CCUGUGUAUA UUGCAAGACA GUAUUG 26

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

CAAUACUGUC UUGCAAUAUA CACAGG 26

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GAACTTACAG AGGTATTTGA ATTTGC 26

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GCAAATTCAA ATACCTCTGT AAGTTC 26

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GAACUUACAG AGGUAUUUGA AUUUGC 26

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

GCAAAUUCAA AUACCUCUGU AAGUUC 26

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CAACCGAGCA CGACAGGAAC GAC 23

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:
GTCGTTCCTG TCGTGCTCGG TTG 23

(2) INFORMATION FOR SEQ ID NO:71:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:
CAACCGAGCA CGACAGGAAC GAC 23

(2) INFORMATION FOR SEQ ID NO:72:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:
GUCGUUCCUG UCGUGCUCGG UUG 23

(2) INFORMATION FOR SEQ ID NO:73:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:
CCAACGACGC AGAGAAACAC AAG 23

(2) INFORMATION FOR SEQ ID NO:74:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

CTTGTGTTTC TCTGCGTCGT TGG 23

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

CCAACGACGC AGAGAAACAC AAG 23

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CUUGUGUUUC UCUGCGUCGU UGG 23

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

CTTACAGAGG TGCCTGCGGT GC 22

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GCACCGCAGG CACCTCTGTA AG 22

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:
CUUACAGAGG UGCCUGCGGU GC 22

(2) INFORMATION FOR SEQ ID NO:80:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:
GCACCGCAGG CACCUCUGUA AG 22

(2) INFORMATION FOR SEQ ID NO:81:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:
GAACTTACAG AGGTGCCTGC GG 22

(2) INFORMATION FOR SEQ ID NO:82:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:
CCGCAGGCAC CTCTGTAAGT TC 22

(2) INFORMATION FOR SEQ ID NO:83:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GAACUUACAG AGGUGCCUGC GG 22

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

CCGCAGGCAC CUCUGUAAGU UC 22

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

CAGGACACAG TGGCTTTTGA C 21

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GTCAAAAGCC ACTGTGTCCT G 21

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

CAGGACACAG UGGCUUUUGA C 21

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

GUCAAAAGCC ACUGUGUCCU G 21

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

GCTTTTTGTC CAGATGTCTT TGC 23

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GCAAAGACAT CTGGACAAAA AGC 23

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

GCUUUUUGUC CAGAUGUCUU UGC 23

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:
GCAAAGACAU CUGGACAAAA AGC 23

(2) INFORMATION FOR SEQ ID NO:93:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:
GCAATGTAGG TGTATCTCCA TGC 23

(2) INFORMATION FOR SEQ ID NO:94:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:
GCATGGAGAT ACACCTACAC CGC 23

(2) INFORMATION FOR SEQ ID NO:95:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:
GCAAUGUAGG UGUAUCUCCA UGC 23

(2) INFORMATION FOR SEQ ID NO:96:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GCAUGGAGAU ACACCUACAC CGC 23

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

AATTTAATAC GACTCACTAT AGGGAGA 27

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

TCTCCCTATA GTGAGTCGTA TTAAATT 27

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

AAUUUAAUAC GACUCACUAU AGGGAGA 27

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

UCUCCCUAUA GUGAGUCGUA UUAAAUU 27

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

TCGTTTTTCA TTAAGGTGTC TAAGTTTTTC TGCTGGATTC 40

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

GAATCCAGCA GAAAAACTTA GACACCTTAA TGAAAAACGA 40

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

UCGUUUUUCA UUAAGGUGUC UAAGUUUUUC UGCUGGAUUC 40

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

GAAUCCAGCA GAAAAACUUA GACACCUUAA UGAAAAACGA 40

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:
GCAATGTTGC CTTAGGTCCA TGC 23

(2) INFORMATION FOR SEQ ID NO:106:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:
GCATGGACCT AAGGCAACAT TGC 23

(2) INFORMATION FOR SEQ ID NO:107:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:
GCAAUGUUGC CUUAGGUCCA UGC 23

(2) INFORMATION FOR SEQ ID NO:108:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:
GCAUGGACCU AAGGCAACAU UGC 23

(2) INFORMATION FOR SEQ ID NO:109:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

CGGTTTCTGG CACCGCAGGC AC 22

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

GTGCCTGCGG TGCCAGAAAC CG 22

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

CGGUUUCUGG CACCGCAGGC AC 22

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

GUGCCUGCGG UGCCAGAAAC CG 22

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

GCAATGTAGC CGTATGTCCA TGC 23

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:
GCATGGACAT ACGGCTACAT TGC 23

(2) INFORMATION FOR SEQ ID NO:115:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:
GCAAUGUAGC CGUAUGUCCA UGC 23

(2) INFORMATION FOR SEQ ID NO:116:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:
GCAUGGACAU ACGGCUACAU UGC 23

(2) INFORMATION FOR SEQ ID NO:117:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:
CACTTCACTG CAAGACATAG AAATAACCTG TGTATATT 38

(2) INFORMATION FOR SEQ ID NO:118:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

AATATACACA GGTTATTTCT ATGTCTTGCA GTGAAGTG 38

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

CACUUCACUG CAAGACAUAG AAAUAACCUG UGUAUAUU 38

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

AAUAUACACA GGUUAUUUCU AUGUCUUGCA GUGAAGUG 38

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

TTATTAATAA GGTGCCTGCG GTGCCAGAAA CC 32

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

GGTTTCTGGC ACCGCAGGCA CCTTATTAAT AA 32

(2) INFORMATION FOR SEQ ID NO:123:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

UUAUUAAUAA GGUGCCUGCG GUGCCAGAAA CC 32

(2) INFORMATION FOR SEQ ID NO:124:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

GGUUUCUGGC ACCGCAGGCA CCUUAUUAAU AA 32

(2) INFORMATION FOR SEQ ID NO:125:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

GACTCTGTGT ATGGAGACAC ATT 23

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

AATGTGTCTC CATACACAGA GTC 23

(2) INFORMATION FOR SEQ ID NO:127:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

GACUCUGUGU AUGGAGACAC AUU 23

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

AAUGUGUCUC CAUACACAGA GUC 23

## Claims

1. A hybridization assay probe comprising an oligonucleotide which will hybridize to an HPV Type 16 and/or Type 18 target nucleic acid to form a detectable target:probe duplex under selective stringency hybridization conditions and which will not form a detectable non-target:probe duplex with nucleic acids from HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, and/or 58 under said conditions;

   wherein said oligonucleotide comprises a sequence of nucleic acids which is at least 70% complementary to a target sequence of 10 or more contiguous nucleotides present in a target region;
   wherein said target region comprises a sequence selected from the group consisting of those set forth in SEQ ID NOs: 9-12, 17-20, 29-32, 33-36, 45-48, and 73-76 or wherein said target region consists of a sequence present in a sequence selected from the group of consisting of those set forth in SEQ ID NOs:5-8, 25-28, 57-60, 65-68, 77-80, and 81-84.

2. The probe of claim 1 which will hybridize to HPV Type 16 nucleic acid, wherein said target region comprises a sequence present in a sequence selected from the group consisting of those set forth in SEQ ID NOs.9-12, 17-20, 29-32, and 33-36 or wherein said target region consists of a sequence present in a sequence selected from the group of consisting of those set forth in SEQ ID NOs: 5-8, and 25-28.

3. The probe of claim 2 which will not hybridize to HPV Type 18 nucleic acid under said conditions.

4. The probe of claim 1 which will hybridize to HPV Type 18 target nucleic acid. wherein said target region comprises a sequence selected from the group consisting of those set forth in SEQ ID NOs: 45-48, and 73-76, or wherein said target region consists of a sequence selected from the group of consisting of those set forth in SEQ ID NOs: 57-60, 65-68, 77-80, and 81-84.

5. The probe of claim 4 which will not hybridize to HPV Type 16 nucleic acid under said conditions.

6. The probe of claim 1, wherein said target region is DNA.

7. The probe of claim 1, wherein said target region is RNA.

8. The probe of claim 1, wherein said target region is selected from the group consisting of those set forth in:

i) SEQ ID NOs: 33-36, and 45-48 or
ii) SEQ ID NOs: 9-12, 17-20, 29-32, and 73-76 or
iii) SEQ ID NOs: 5-8, 25-28, 57-60, 65-68, 77-80, 81-84, and 85-88.

9. The probe of claim 1 which hybridizes to nucleic acid of HPV Type 16 and/or Type 18 and not to HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, and/or 58 at 50 to 60°C in 0.04M to 0.06M lithium succinate buffer containing between 0.9 and 1.1% lithium lauryl sulfate, wherein said duplex is stable for the detection of HPV Type 16 and/or Type 18 and not for the detection of HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, and/or 58.

10. The probe of claim 1, wherein said selective stringency hybridization conditions comprise 0.10M to 0.14M phosphate buffer containing approximately equimolar amounts of $Na_2HP0_4$ and $NaH_2P0_4$, approximately 1 mM EDTA, and 0.01 to 0.03% sodium dodecyl sulfate at 60 to 70°C.

11. The probe of claim 1, wherein said oligonucleotide comprises a sequence which is at least 90%, preferably 100%, complementary to said target sequence of 10 or more contiguous nucleotides.

12. The probe of claim 1 wherein said oligonucleotide is 10 to 100, preferably 15 to 50, bases in length.

13. The probe of claim 1, wherein said oligonucleotide is 23-40 bases in length.

14. A nucleic acid hybrid formed between a target nucleic acid sequence of 10 or more contiguous nucleic acids present in a target region of anyone of claims 1-13 and a corresponding oligonucleotide of anyone of claims 1-12 wherein said hybrid is stable for the detection of HPV Type 16 and/or Type 18 and not for the detection of HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, and/or 58.

15. The nucleic acid hybrid of claim 14 further comprising a site for the initiation of nucleic acid synthesis and wherein under stringent hybridization conditions said oligonucleotide hybridizes specifically to nucleic acid of HPV Type 16 and/or Type 18 and not to HPV Types 6, 11, 31, 33, 35, 39, 45, 51, 52, or 58.

16. A helper probe comprising an oligonucleotide, wherein said oligonucleotide comprises a sequence of nucleotides which will hybridize to a HPV Type 16 and/or Type 18 target sequence
wherein said target sequence is selected from the group consisting of those set forth in SEQ ID NOs: 62, 64, 118, 120, 122, 124, 126 and 128.

17. The probe of claim 16, wherein said oligonucleotide is DNA.

18. The probe of claim 16, wherein said oligonucleotide is RNA.

19. The probe of claim 16, wherein said target sequence comprises a sequence selected from the group consisting of those set forth in:

i) SEQ ID NOs: 126, and 128 or
ii) SEQ ID NOs: 62, 64, 122, and 124 or
iii) SEQ ID NOs: 118, and 120.

20. The probe of claim 16, wherein said oligonucleotide is at least 90%, preferably 100%, identical to 10 or more contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOs: 61, 63, 117, 119, 121, 123, 125, and 127.

21. The probe of claim 16 wherein said oligonucleotide is 10 to 100, preferably 15 to 50, most preferably 23-40 bases in length.

22. A probe mix comprising a combination of; (1) one or more nucleic acid hybridization assay probes, and (2) one or more amplification oligonucleotides, and/or (3) one or more helper probes wherein said combination is selected from the group consisting of:

(a) one or more hybridization assay probes, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 5 or 6 and one or more amplification oligonucleotides, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 1 or 85;
(b) one or more hybridization assay probes, at least one of, which has a nucleic acid sequence substantially similar to SEQ ID NO: 33 or 34 and one or more amplification oligonucleotides, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 37, 93, or 113;
(c) one or more hybridization assay probes, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 9 or 10 and one or more amplification oligonucleotides, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 21 or 89;
(d) one or more hybridization assay probes, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 17, 18, 25, 26, 29, or 30 and one or more amplification oligonucleotides, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 13 or 21;
(e) one or more hybridization assay probes at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 45 or 46 and one or more amplification oligonucleotides at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 53 or 109;
(f) one or more hybridization assay probes, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 73 or 74 and one or more amplification oligonucleotides, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 113 or 69; and
(g) one or more hybridization assay probes, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 65, 66, 81, or 82 and one or more amplification oligonucleotides, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 41 or 109;
(h) one or more hybridization assay probes, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 65, 66, 81, or 82 and one or more amplification oligonucleotides, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 41 or 109, and one or more helper probes, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 61, 63, 117, or 119; and
(i) one or more hybridization assay probes, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 5, 6, 7, 8, 45, 46, 47, 48, or 49 and one or more helper probes, at least one of which has a nucleic acid sequence substantially similar to SEQ ID NO: 121, 123, 125, or 127.

**23.** An amplification oligonucleotide for amplifying HPV Type 16 and/or Type 18 nucleic acid sequences;

wherein said oligonucleotide comprises a sequence of nucleotides which has a region that is at least 70% complementary to a subsequence of 10 or more contiguous nucleotides present in a target sequence,
wherein said target sequence comprises a sequence selected from the group consisting of those set forth in SEQ ID NOs: 2, 4, 14, 16, 22, 24, 38, 40, 42, 44, 50, 52, 54, 56, 70, 72, 90, 92, 94, 96, 102, 104, 106, 108, 110, 112, 114 and 116, or consists of a sequence selected from the group consisting of those set forth in SEQ ID NOs: 86 and 88.

**24.** The oligonucleotide of claim 23, wherein said oligonucleotide is DNA.

**25.** The oligonucleotide of claim 23, wherein said oligonucleotide is RNA.

**26.** The oligonucleotide of claim 23, wherein said target sequence comprises a sequence selected from the group of consisting of those set forth in:

i) SEQ ID NOs: 38, 40, 94, 96, 102, and 104 or
ii) SEQ ID NOs: 2, 4, 10, 12, 14, 16, 22, 24, 50, 52, 54, 56, 70, 72, 90, 92, 106, 108, 110, 112, 114, and 116 or iii) SEQ ID NOs: 42, 44, 86, and 88.

**27.** The oligonucleotide of claim 23, wherein said oligonucleotide is at least 90%, preferably 100%, identical to a subsequence of 10 or more contiguous nucleotides present in sequence selected from the group consisting of those set forth in SEQ ID NOs: 1, 3, 13, 15, 21, 23, 37, 39, 41, 43, 49, 51, 53, 55, 69, 71, 85, 87, 89, 91, 93, 95, 101, 103, 105, 107, 109, 111, 113 and 115.

**28.** The oligonucleotide of claim 23 wherein said oligonucleotide is 10 to 100, preferably 15 to 50, most preferably 23-40 bases in length.

**29.** A method for selectively amplifying HPV Type 16 and/or Type 18 nucleic acid in a sample, comprising the step of

amplifying said nucleic acid with one or more amplification oligonucleotides of anyone of claims 23-28.

30. A method for detecting HPV Type 16 and/or Type 18 in a sample potentially containing said HPV Type 16 and/or Type 18 comprising the steps of:

a) providing to said sample one or more nucleic acid hybridization assay probes of anyone of claims 1-15; and
b) detecting the formation of said detectable probe:target duplex which is indicative of the presence of HPV Type 16 and/or Type 18.

31. The method of claim 30, wherein said target nucleic acid is amplified with a probe of anyone of claims 23-28 and detected with a probe of anyone of claims 1-12.

32. A kit comprising one or more probes of anyone of claims 1-12.

33. A method of specifically detecting a HPV Type 16 nucleic acid in preference to a HPV Type 18 nucleic acid in a sample comprising the steps of:

a) amplifying said HPV Type 16 nucleic acid with at least one amplification oligonucleotide which will bind to or cause extension through an HPV Type 16 nucleotide sequence selected from the groups consisting of:

(i) SEQ ID NO: 2 and 4; and
(ii) SEQ ID NO: 86 and 88

b) contacting amplified HPV Type 16 nucleic acids with at least one hybridization assay probe which will form a detectable probe: target hybrid under stringent hybridization conditions with at least a portion of nucleotide sequence target region of HPV 16 selected from the group consisting of:

(i) SEQ ID NO: 6 and SEQ ID NO: 8; and
(ii) SEQ ID NO: 5 and SEQ ID NO: 7

c) imposing stringent hybridization conditions, and
d) detecting the presence of probe: target hybrid as an indication of the presence or amount of HPV Type 16 in said sample.

34. The method of claim 33, wherein said amplification oligonucleotide has a nucleotide sequence substantially similar to SEQ ID NO: 1 or 85.

35. The method of claim 34, wherein said probe has nucleotide sequence substantially similar to SEQ ID NO: 5 or 6.

36. A method of specifically detecting a HPV Type 16 nucleic acid in preference to a HPV Type 18 nucleic acid in a sample comprising:

a) amplifying said HPV Type 16 nucleic acid with at least one amplification oligonucleotide which will bind to or cause extension through an HPV Type 16 nucleotide sequence selected from the groups consisting of:

(i) SEQ ID NO: 38 and 40;
(ii) SEQ ID NO: 94 and 94; and
(iii) SEQ ID NO: 114 and 116

b) contacting amplified HPV Type 16 nucleic acids with at least one hybridization assay probe which will form a detectable probe: target hybrid under stringent hybridization conditions with at least a portion of a nucleotide sequence target region of HPV 16 selected from the group consisting of:

(i) SEQ ID NO: 34 and SEQ ID NO: 36; and
(ii) SEQ ID NO: 33 and SEQ ID NO: 35

c) imposing stringent hybridization conditions, and
d) detecting the presence of probe: target hybrid as an indication of the presence or amount of HPV Type 16

in said sample.

37. The method of claim 36, wherein said amplification oligonucleotide has a nucleotide sequence substantially similar to SEQ ID NO: 37, 93, or 113.

38. The method of claim 36, wherein said probe has a nucleotide sequence substantially similar to SEQ ID NO: 33 or 34.

39. A method of specifically detecting a HPV Type 16 nucleic acid in preference to a HPV Type 18 nucleic acid in a sample comprising:

a) amplifying said HPV Type 16 nucleic acid with at least one amplification oligonucleotide which will bind to or cause extension through an HPV Type 16 nucleotide sequence selected from the groups consisting of:

(i) SEQ ID NO: 90 and 92; and
(ii) SEQ ID NO: 22 and 24

b) contacting amplified HPV Type 16 nucleic acids with at least one hybridization assay probe which will form a detectable probe: target hybrid under stringent hybridization conditions with at least a portion of a nucleotide sequence target region of HPV 16 selected from the group consisting of:

(i) SEQ ID NO: 9 and SEQ ID NO: 11; and
(ii) SEQ ID NO: 10 and SEQ ID NO: 12

c) imposing stringent hybridization conditions, and
d) detecting the presence of probe: target hybrid as an indication of the presence or amount of HPV Type 16 in said sample.

40. The method of claim 39, wherein said amplification oligonucleotide has a nucleotide sequence substantially similar to SEQ ID NO: 21 or 89.

41. The method of claim 39, wherein said probe has nucleotide sequence substantially similar to SEQ ID NO: or 10.

42. A method of specifically detecting a HPV Type 16 nucleic acid in preference to a HPV Type 18 nucleic acid in a sample comprising:

a) amplifying said HPV Type 16 nucleic acid with at least one primer which will bind to or cause extension through an HPV Type 16 nucleotide sequence selected from the groups consisting of:

(i) SEQ ID NO: 22 and 24; and
(ii) SEQ ID NO: 14 and 16
b) contacting amplified HPV Type 16 nucleic acids with at least one oligonucleotide probe which will form a detectable probe: target hybrid under stringent hybridization conditions with at least a portion of a nucleotide sequence target region of HPV 16 selected from the group consisting of:

(i) SEQ ID NO: 30 and SEQ ID NO: 32;
(ii) SEQ ID NO: 26 and SEQ ID NO: 28; and
(iii)SEQ ID NO: 18 and SEQ ID NO: 20

c) imposing stringent hybridization conditions, and
d) detecting the presence of probe: target hybrid as an indication of the presence or amount of HPV Type 16 in said sample.

43. The method of claim 42, wherein said amplification oligonucleotide has a nucleotide sequence substantially similar to SEQ ID NO: 13 or 21.

44. The method of claim 42, wherein said probe has a nucleotide sequence substantially similar to SEQ ID NO: 17, 18, 25, 26, 29, or 30.

45. The method of claim 42, wherein said probe has a nucleotide sequence substantially similar to SEQ ID NO: 29 or 30 and said amplified Type 16 nucleic acids have a sequence-specific to unspliced E6 mRNA or its complement, and DNA versions thereof having thymine substituted for uracil.

46. The method of claim 42, wherein said probe has a nucleotide sequence substantially similar to SEQ ID NO: 25 or 26 and said amplified Type 16 nucleic acids have a sequence specific to spliced E6* mRNA or its complement, and DNA versions thereof having thymine substituted for uracil.

47. The method of claim 42, wherein said probe has a nucleotide sequence substantially similar to SEQ ID NO: 17 or 18 and said amplified Type 16 nucleic acids have a sequence specific to E6** mRNA or its complement, and DNA versions thereof having thymine substituted for uracil.

48. A method of specifically detecting a HPV Type 18 nucleic acid in preference to a HPV Type 16 nucleic acid in a sample comprising the steps of:

a) amplifying said HPV Type 18 nucleic acid with at least one amplification oligonucleotide which will bind to or cause extension through an HPV Type 18 nucleotide sequence selected from the groups consisting of:

(i) SEQ ID NO: 54 and 56; and
(ii) SEQ ID NO: 110 and 112

b) contacting amplified HPV Type 18 nucleic acids with at least one hybridization assay probe which will form a detectable probe: target hybrid under stringent hybridization conditions with at least a portion of nucleotide sequence target region of HPV 18 selected from the group consisting of:

(i) SEQ ID NO: 46 and SEQ ID NO: 48; and
(ii) SEQ ID NO: 45 and SEQ ID NO: 47

c) imposing stringent hybridization conditions, and
d) detecting the presence of probe: target hybrid as an indication of the presence or amount of HPV Type 18 in said sample.

49. The method of claim 48, wherein said amplification oligonucleotide has a nucleotide sequence substantially similar to SEQ ID NO: 53 or 109.

50. The method of claim 48, wherein said probe has a nucleotide sequence substantially similar to SEQ ID NO: 45 or 46.

51. A method of specifically detecting a HPV Type 18 nucleic acid in preference to a HPV Type 16 nucleic acid in a sample comprising:

a) amplifying said HPV Type 18 nucleic acid with at least one amplification oligonucleotide which will bind to or cause extension through an HPV Type 18 nucleotide sequence selected from the groups consisting of:

(i) SEQ ID NO: 70 and 72;
(ii) SEQ ID NO: 38 and 30; and
(iii)SEQ ID NO: 114 and 116

b) contacting amplified HPV Type 18 nucleic acids with at least one hybridization assay probe which will form a detectable probe: target hybrid under stringent hybridization conditions with at least a portion of a nucleotide sequence target region of HPV 18 selected from the group consisting of:

(i) SEQ ID NO: 74 and SEQ ID NO: 76; and
(ii) SEQ ID NO: 73 and SEQ ID NO: 75

c) imposing stringent hybridization conditions,and
d) detecting the presence of probe: target hybrid as an indication of the presence or amount of HPV Type 18 in said sample.

52. The method of claim 51, wherein said amplification oligonucleotide has a nucleotide sequence substantially similar to SEQ ID NO: 113 or 69.

53. The method of claim 51, wherein said probe has a nucleotide sequence substantially similar to SEQ ID NO: 73 or 74.

54. A method of specifically detecting a HPV Type 18 nucleic acid in preference to a HPV Type 16 nucleic acid in a sample comprising:

a) amplifying said HPV Type 18 nucleic acid with at least one amplification oligonucleotide which will bind to or cause extension through an HPV Type 18 nucleotide sequence selected from the groups consisting of:

(i) SEQ ID NO: 42 and 44; and
(ii) SEQ ID NO: 110 and 112

b) contacting amplified HPV Type 18 nucleic acids with at least one hybridization assay probe which will form a detectable probe: target hybrid under stringent hybridization conditions with at least a portion of a nucleotide sequence target region of HPV 18 selected from the group consisting of:

(i) SEQ ID NO: 82 and SEQ ID NO: 84;
(ii) SEQ ID NO: 66 and SEQ ID NO: 68; and
(iii) SEQ ID NO: 56 and SEQ ID NO: 58

c) imposing stringent hybridization conditions, and
d) detecting the presence of probe: target hybrid as an indication of the presence or amount of HPV Type 18 in said sample.

55. The method of claim 54, wherein said amplification oligonucleotide has a nucleotide sequence substantially similar to SEQ ID NO: 41 or 109.

56. The method of claim 54, wherein said probe has a nucleotide sequence substantially similar to SEQ ID NO: 65, 66, 81, or 82.

57. The method of claim 54, wherein said probe has a nucleotide sequence substantially similar to SEQ ID NO: 65 or 66 and said amplified Type 18 nucleic acids have a sequence specific to unspliced E6 mRNA or its complement, and DNA versions thereof having thymine substituted for uracil.

58. The method of claims 54, wherein said probe has a nucleotide sequence substantially similar to SEQ ID NO: 81 or 82 and said amplified Type 18 nucleic acids have a sequence specific to spliced E6* mRNA or its complement, and DNA versions thereof having thymine substituted for uracil.

59. The method of claim 54, wherein said probe has a nucleotide sequence substantially similar to SEQ ID NO: 57 or 58 and said amplified Type 16 nucleic acids have a sequence specific to E6** mRNA or its complement, and DNA versions thereof having thymine substituted for uracil.

60. The method of claim 54 comprising providing to said sample a helper probe comprising an oligonucleotide, wherein said oligonucleotide comprises a sequence of nucleotides which will hybridize to a HPV Type 16 and/or 18 target sequence,
wherein said target sequence is selected from the group consisting of those set forth in SEQ ID NOs: 62, 64, 118, and 120.

61. The method of any of claims 31 to 60 wherein at least one amplification oligonucleotide further has a 5' portion able to promote initiation of RNA transcription.

62. The method of claim 61, wherein said 5' portion comprises a nucleotide sequence substantially similar to SEQ ID NO: 97.

63. A method of specifically detecting a HPV Type 16 or HPV Type 18 nucleic acid in preference to a HPV Type 6, 11, 31, 33, 35, 39, 45, 51, 52, or 58 nucleic acid in a sample comprising:

a) contacting HPV Type 16 nucleic acid with at least one hybridization assay probe which will form a detectable probe: target hybrid under stringent hybridization conditions with at least a portion of a nucleotide sequence target region of HPV 16 selected from the group consisting of:

(i) SEQ ID NO: 6 and SEQ ID NO: 8; and
(ii) SEQ ID NO: 5 and SEQ ID NO: 7;

b) contacting amplified HPV Type 18 nucleic acids with at least one hybridization assay probe which will form a detectable probe: target hybrid under stringent hybridization conditions with at least a portion of a nucleotide sequence target region of HPV 18 selected from the group consisting of:

(i)SEQ ID NO: 46 and SEQ ID NO: 48;and
(ii) SEQ ID NO: 45 and SEQ ID NO: 47;

c) imposing stringent hybridization conditions, and
d) detecting the presence of probe: target hybrid as an indication of the presence or amount of HPV Type 16 or HPV Type 18 in said sample.

**64.** The method of claim 63 comprising providing to said sample a helper probe comprising an oligonucleotide having a sequence substantially similar to SEQ ID NO: 121, 123, 125, or 127.

**65.** A method of specifically detecting a HPV type 18 nucleic acid in preference to an HPV type 16 nucleic acid in a sample comprising:

a) amplifying said HPV type 18 nucleic acid with at least one amplification oligonucleotide which will bind to or cause extension through an HPV type 18 nucleotide sequence selected from the groups consisting of

(i) SEQ ID NO: 42 and 44, or
(ii) SEQ ID NO: 110 and 112,

b) contacting amplified HPV type 18 nucleic acids with at least one hybridization assay probe which will form a detectable probe:target hybrid under stringent hybridization conditions with at least a portion of a nucleotide sequence target region of HPV 18 selected from the group consisting of

(i) SEQ ID NO: 81 and 83, for HPV 18 E6
(ii) SEQ ID NO: 65 and 67, for HPV 18 E6* and
(iii) SEQ ID NO: 57 and 59, for HPV 18 E6** ;

c) imposing stringent hybridization conditions
d) detecting the presence of probe:target hybrid as an indication of the presence or amount of HPV type 18 in said sample.

**66.** The method of claim 65 wherein said primer has a nucleotide sequence substantially similar to SEQ ID NO: 41 or 109.

**67.** The method of claim 65 wherein at least one primer further has a 5' portion able to promote RNAS transcription and/or comprising a nucleotide sequence substantially similar to SEQ ID NO: 97.

**68.** The method of claim 65 wherein said probe has a nucleotide sequence substantially similar to a sequence selected from the group consisting of SEQ ID NO: 81, 57, or 65.

**69.** The method of claim 65 comprising providing to said sample a helper probe comprising an oligonucleotide having a sequence substantially similar to SEQ ID NO: 61, 117.

**70.** A kit comprising: (a) one or more hybridization assay probes of anyone of claims 1-12; and (b) one or more amplification oligonucleotides of anyone of claims 23-28.

**71.** A kit comprising: (a) one or more hybridization assay probes of anyone of claims 1-12; and (b) one or more helper

probes of anyone of claims 16-21.

**72.** A kit comprising a probe mix of claim 22.